









LECTURES ON
PLANT PATHOLOGY
AND PHYSIOLOGY IN
RELATION TO MAN

A SERIES OF LECTURES GIVEN AT THE MAYO
FOUNDATION AND THE UNIVERSITIES OF MINNE-
SOTA, IOWA, WISCONSIN, THE DES MOINES ACAD-
EMY OF MEDICINE, IOWA, AND IOWA STATE
COLLEGE

1926-1927

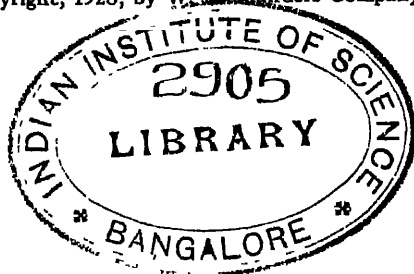


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INTRODUCTION

This group of lectures on plant pathology and physiology in relation to man was given during the autumn and winter of 1926 and 1927 under the auspices of The Mayo Foundation and the local chapter of Sigma Xi at Rochester, Minnesota, the Medical School of the University of Wisconsin, Madison, Wisconsin, the Graduate School of the University of Minnesota, Minneapolis, Minnesota, the Graduate School of the University of Iowa, Iowa City, Iowa, the Iowa State College, Ames, Iowa, and the Des Moines Academy of Medicine, Des Moines, Iowa

The lectures cover many important aspects of recent investigation in the field of plant pathology and physiology. The lecturers themselves were chosen because of their original research work in the fields concerned. The volume thus presents in an authoritative manner many important problems of interest not only to plant pathologists, but to biologists, chemists, physiologists, pathologists, and physicians.

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Director

ROCHESTER, MINNESOTA.
November, 1928

The Mayo Foundation for
Medical Education and Research

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Lectures on Plant Pathology and Physiology in Relation to Man

FILTERABLE VIRUSES*

LOUIS OTTO KUNKEL

Many infectious diseases of plants are caused by bacteria and fungi. A few are caused by myxomycetes and flagellates. Pear blight was the first plant disease shown to be due to bacteria. Its cause was discovered by Burrill about forty-five years ago. Fungi were first proved to cause plant diseases about the middle of the nineteenth century. The well known club root of cabbage was the first plant disease shown to be due to a myxomycete. The causal organism was discovered by Woronin in 1878. The first latex inhabiting plant flagellate was discovered by Lafont in 1909. It will be seen that the discoveries which first proved that members of these four groups of organisms cause infectious plant diseases are of

* Lecture given before the State University of Iowa, Iowa City, January 11, 1927, Iowa State College, Ames, Iowa, January 12, Academy of Medicine, Des Moines, Iowa, January 12, University of Minnesota, Minneapolis, January 13, and The Mayo Foundation, Rochester, Minnesota, January 14

comparatively recent dates. Nevertheless the parasitic activities of these groups are now fairly well understood. There are, however, many infectious plant diseases which are apparently not due to organisms belonging to either of these groups. These diseases are said to be caused by viruses. Many of the viruses pass bacteria-proof filters and are frequently referred to as filterable viruses. It is with this group of plant maladies that the present paper is concerned.

The virus diseases of plants are, like other plant diseases, important to man because they attack the economic species of plants which produce materials used for food, clothing, and shelter. They are also important in another way. They are similar to many little understood diseases that attack man and animals. If we could discover the cause of any one of the virus diseases of plants it would doubtless furnish the key to an understanding of other similar diseases. The list of virus diseases of man and animals is a long one. I shall mention only a few that are well known. Measles, smallpox, and typhus fever are some of the most dreaded of the virus diseases of man. It is believed by some that cancer may also belong in this group. As examples of virus diseases of animals I shall mention only rabies, hog cholera, and the foot-and-mouth disease of cattle. The students of the pathology of man and animals have learned many important facts regarding these diseases, but the nature of the agent causing them is unknown. As long as the causative entity remains unknown we are under a great handicap in efforts to control, eradicate, or prevent the spread of these diseases.

In the study of virus diseases the plant pathologist has certain well known advantages. There are so many different virus diseases of plants that he has a wide range in choice of material for study. Because plants are easy to grow, they can be used in relatively large numbers in experimental work. The environmental conditions under which plants live are more readily controlled than in the case of animals. Plants have a simple anatomic structure and can be dissected and studied in the living condition. For these and other reasons plants are especially suitable organisms in which to study virus diseases. Considering the great importance of this group of plant maladies to agriculture and considering the close relationship between virus diseases of plants and some of the most dreaded diseases of man and animals it is not surprising that plant pathologists are devoting much attention to the virus disease problem. Many important facts have already been learned regarding a number of these diseases. To a certain extent, some of them are being controlled. But their true nature and etiology is an unsolved problem.

In a report dated April 17, 1897 but not published until March, 1898, Loeffler and Frosch showed that the agent causing foot-and-mouth disease of cattle and other animals is capable of passing through fine porcelain filters which hold back ordinary bacteria. They also showed that the agent passing these filters can cause the foot-and-mouth disease after successive transfers through susceptible animals. They thus proved that the agent differs from bacteria on the one hand and from poisonous substances on the other. They showed for the first time the existence of an infectious disease

due to a filter-passing agent. They suggested that the agent might be an organism too small to come within the range of microscopic vision.

In 1886 Mayer described a tobacco disease to which he gave the name "mosaic." He showed that the juice from plants having this disease is capable of infecting healthy plants and expressed the opinion that the disease might be due to a bacterium. In 1898 Beijerinck showed that juice from mosaic tobacco plants may be passed through porcelain filters without losing the agent causing the disease. He was aware that the causative entity rapidly increases in diseased plants. He found that the quantity of filtered juice necessary to infect plants is extremely small but that with the juice obtained from the leaves of plants so infected and in which the disease later developed, he could infect an almost unlimited number of healthy plants. Beijerinck was impressed with the infectiousness of mosaic disease, with the rapidity with which the causative agent increases in diseased plants, and with the ability of this agent to pass bacterium-proof filters. He was, therefore, convinced that the causative agent could not be an ordinary bacterium and he stated his hypothesis of a "contagium vivum fluidum." He predicted that other plant diseases would be found which, like tobacco mosaic, may be caused by a "contagium fluidum" and stated that peach yellows which had been described by Smith undoubtedly belongs in this group.

Most of the conclusions reached by Beijerinck were confirmed by Iwanowski, who made a careful study of the tissues of mosaic tobacco plants. In his search for a bacterium which

he thought might be present in the tissues or in cells of diseased plants he discovered plastic ameboid intracellular bodies which are never found in the cells of healthy tobacco plants. He noted the similarity between these bodies and certain amebæ and considered the possibility that the bodies might be of etiologic significance. Since, however, the agent causing tobacco mosaic can pass through the pores of a porcelain filter he concluded that the ameboid bodies must be a result rather than a cause of the disease. During the same year that Iwanowski published his paper describing ameboid bodies associated with tobacco mosaic, Negri reported small bodies in the brain cells of animals suffering from rabies. In 1910 Lyon described inclusions that are associated with the Fiji disease of sugar cane. These inclusions resemble Negri bodies. Bodies similar to those described by Negri have been shown to be associated with many of the virus diseases of man and animals. In each case the bodies are characteristic of the diseases with which they are associated and are of considerable diagnostic value. The ameboid bodies described by Iwanowski or other similar bodies were apparently not observed by those who studied mosaic and other virus diseases of plants during a period of eighteen years following the publication of Iwanowski's paper. No mention is made of such bodies in the literature of this period. In cytologic and histologic studies on the corn mosaic of Hawaii published in 1921 Kunkel described and pictured ameboid intracellular bodies that are associated with this disease. The bodies are present in almost all of the cells in the chlorotic portions of diseased corn leaves but are not present in the cells of the

green portions of such leaves or in the cells of healthy plants. They also occur in the cells of diseased stalk tissues.

Much attention has recently been given to the characteristic ameboid bodies that are now known to be associated with a considerable number of different virus diseases of plants. The bodies originally described by Iwanowski in the cells of mosaic tobacco leaves have recently been studied by Rawlins and Johnson, Goldstein, and others. These bodies have been given special attention because of the belief that they may be of etiologic significance. They are similar to the bodies associated with the virus diseases of animals and serve to emphasize the close relationship existing between these diseases and the virus diseases of plants.

Many different opinions are held as to the nature of the bodies. Some believe them to be stages in the life cycle of a parasitic organism. Others think them to be degeneration products resulting from the disease. All that can safely be said at the present time is that they are vacuolate ameboid bodies having a structure similar to that of protoplasm. They may be stages in the life cycle of a parasitic organism, but proof of this is still lacking. Until they have been observed to show vital movement, have been demonstrated to possess nuclei, have been cultivated on artificial mediums or have been proved by their morphology or life history to be related to some known organism, we shall have to reserve judgment as to whether or not they are living.

I have stated that the etiology of the virus diseases of both plants and animals is an unsolved problem. But the situation is even worse than this statement indicates. At the pres-

ent time there is no clear understanding as to what observations and experiments should be made in order to solve the virus disease mystery. All that can be done is to study each disease as carefully and as thoroughly as possible, using the best technic at present available. Any evidence that these diseases are or are not due to living organisms is important.

The number of virus diseases from which to choose material for study is very large. It would be useless to attempt to give even an approximately complete list of the virus diseases of plants. Every student of these diseases knows that many of them have not yet been described. A few of the virus diseases that attack plants of some economic importance and have been more or less carefully studied will be mentioned. The virus diseases of plants are known to be spread in five different ways; by means of juice from diseased plants, by grafts, by seeds, by cuttings, and by insects. Since cuttings are really detached parts of the plants from which they are taken the spread of these diseases by cuttings is not a means of transmission to new plants. Spread by cuttings is included here because of its importance in the case of crops such as potatoes and sugar cane that are propagated by cuttings.

All virus diseases of plants are infectious. Some, like cucumber mosaic and tobacco mosaic, are transmitted with the greatest readiness. Others, like curly-top of beets and sugar cane mosaic, are transmitted mechanically with considerable difficulty. Still others, such as Abutilon mosaic, peach yellows, and spike of sandal, have been transmitted only by grafting. Some, as for example false blossom of cranberries and wheat rosette, have not yet been experimentally transmitted. They

are included in this list because they show symptoms characteristic of virus diseases.

So far as is known, all virus diseases of plants are systemic but in certain cases they remain local for some time after infection. The causative entity apparently spreads to all parts of infected plants, although its effects may differ in different tissues. Marked macroscopic symptoms appear only in parts that develop after plants are infected. The virus diseases are frequently given names that suggest some conspicuous symptom. Yellow diseases, such as aster yellows and peach yellows, cause chlorosis throughout the green portions of diseased plants. In the mosaic diseases the light and dark green tissues are so distributed as to produce a mosaic. The name given to spike disease of sandal emphasizes the upright habit of growth characteristic of the disease. This symptom results from the effect of the disease on the response of plants to gravity. The main stems of most normal plants grow upright. The secondary shoots branch off in such a way as to make an angle with the vertical which is characteristic for each species. The secondary shoots produced by plants having such diseases as aster yellows, peach yellows, false blossom of cranberry, and spike of sandal show a more upright habit of growth than do the secondary shoots of healthy plants of these species.

Abnormal production of secondary shoots is another common symptom of virus diseases. This is shown conspicuously by the stunt disease of rice, wheat rosette, and the sereh disease of sugar cane. Several different virus diseases cause overgrowths. This is strikingly shown by the Fiji disease of

sugar cane which is a systemic gall disease. The galls are distributed in the phloem tissues throughout the leaves and stalks of infected plants. Although many virus diseases cause stunting and malformation of leaves as well as necrosis they do not in most cases kill plants. The losses caused result from reduction in yield due to chlorosis, necrosis, overgrowths, and other effects of these diseases.

It is fortunate that the virus diseases of plants are not transmitted through seeds except in a few cases. This makes control much easier than it would be if they were regularly seed borne. It also delays the spread of the diseases to new areas. In the case of perennial plants or plants propagated by means of grafts or cuttings control is difficult, for plants once diseased seldom recover, and we have no way of curing them. In only one instance has a means been found of obtaining healthy plants from diseased cuttings. When cuttings from sugar cane plants having sereh disease are held at 50° to 52° C for half an hour, the virus causing this disease is destroyed without killing the cuttings.¹⁴

The means by which many common virus diseases are transmitted to healthy plants under natural condition has not yet been discovered. We do not know how Abutilon mosaic, false blossom of cranberry, peach rosette, peach yellows, spike of sandal, sereh and the Fiji disease of sugar cane and many other virus diseases are spread. Our present knowledge indicates that many of them may be insect borne. The virus diseases that have been most carefully studied are known to be spread by insects.

Takami proved that the stunt or mosaic disease of rice is

transmitted by the leafhopper *Nephotettix apicalis* Motsch. He published the results of his studies in 1901, and was the first to show that a plant virus disease is insect borne. During the past twenty-five years the insect carriers of many other virus diseases have been studied. In some instances the transmission of these diseases by insects seems to be a simple mechanical transfer, but a specific relationship undoubtedly exists between some virus diseases and their insect carriers. Takami found that although the stunt disease of rice is readily transmitted by *Nephotettix apicalis* it is not spread by other rice leafhoppers. The curly-top of sugar beets is transmitted by the leafhopper *Eutettix tenellus* Baker, but not by other sugar beet insects. Grass mosaic is transmitted to sugar cane by the corn aphid, but is not transmitted by *Aphis sacchari* Zehntn. and other sucking insects. The streak disease of corn is transmitted by the leafhopper *Balclutha mbila* Naude, but is not transmitted by the corn leafhopper *Peregrinus maidis* Ashmead which transmits the Hawaiian mosaic disease of corn. Aster yellows is readily spread by the leafhopper *Cicadula sexnotata* Fall. but is not transmitted by several other aster insects including leafhoppers and aphids.

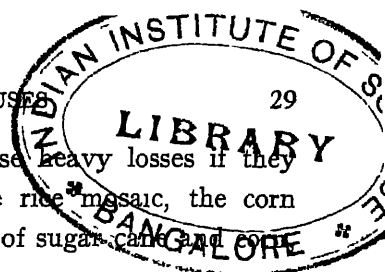
One of the most interesting facts that have been learned in studies on the relation of *Cicadula sexnotata* to the aster yellows disease is that this disease passes an incubation period in the insect. It has been known for some time that the beet leafhopper is unable to transmit curly-top of beets immediately after feeding on diseased plants for the first time. A period varying from a few hours to more than a day must

elapse before this insect is capable of spreading the curly-top disease. In the case of aster yellows the interval between the time when the virus is first obtained and the time when it can be transmitted by the leafhopper to healthy plants is much longer than was found for the sugar beet curly-top disease. The minimal incubation period found for aster yellows in the aster leafhopper is ten days. While the period varies in different individual insects and in different insect colonies the average incubation period is about two weeks. During this time virus carrying insects are unable to transmit the disease. In this respect aster yellows is similar to malaria which passes an incubation period in the body of the mosquito, *Anopheles*. The length of the incubation period is also similar. We know that the period of incubation of malaria in the mosquito results from the development of certain stages in the life cycle of the malaria organism in the body of the mosquito. We do not yet know why aster yellows requires an incubation period in its insect carrier, but the fact that the period exists and is similar to that of some well known parasites suggests that the agent causing aster yellows is a living organism which passes through certain stages of its life cycle in the body of the leafhopper *Cicadula sexnotata*. In spite of careful studies in both the aster plant and the aster leafhopper no organism has yet been found. So far as can be determined virus carrying leafhoppers suffer no ill effects from the virus which they carry and transmit. Nevertheless, the relation of aster yellows to its insect carrier is believed to bring the best evidence which we now possess that virus diseases are due to living organisms. It is not easy to believe

that an autocatalyst, an enzyme, or any other chemical non-living substance would require an incubation period in an insect vector. In the relation of aster yellows to its insect carrier we find a possible explanation of our inability to transmit this disease mechanically except by grafting. Perhaps the stages of the causative entity which develop in the plant are too delicate or are otherwise unsuited for transmission directly from plant to plant. Perhaps only those stages that develop in the insect are capable of transmission to plants.

The specific relationship existing between aster yellows and its insect carrier suggests an explanation of the difficulty which has been experienced in finding the means by which a number of the virus diseases of plants are spread. Many virus diseases are known to spread, but the means by which they spread are unknown. Each of these diseases may be carried by a single insect species which, like the aster leafhopper, may be considered alternate hosts of the disease producing entities which are transmitted. The problem is to find in each case the particular insect concerned. We shall not understand their spread or be in position to effectively control these diseases until we know their insect relationships.

Evidence that insects may be alternate hosts of some of these obscure diseases emphasizes the importance of keeping out of the country all insect species that have not already gained entrance. An insect that is of little importance so far as feeding habits are concerned, may be of great importance in the spread of some virus disease which is already here or may later reach the country. We do not know how many virus diseases are present in other countries. Some that have not



yet reached us and that might cause heavy losses if they should accidentally gain entrance are rice mosaic, the corn mosaic of Hawaii, the streak disease of sugar cane and the sereh and Fiji disease of sugar cane.

I have stated that studies on aster yellows have strengthened the belief that virus diseases are due to living organisms. If, however, this theory is correct, how is our failure to discover a causative organism to be explained. It is, of course, possible that the intracellular ameboid bodies associated with so many of the mosaic diseases of plants represent stages in the life cycles of living organisms. Perhaps the chief reasons for doubting this hypothesis are that the bodies apparently are not associated with all of these diseases and that many of the viruses attacking both plants and animals are known to be filterable.

Loeffler and Frosch long ago suggested that the agent causing foot-and-mouth disease of cattle may be a microbe too small to come within the range of microscopic vision. The hypothesis that ultramicroscopic bacteria may cause the virus diseases has long been popular. It is well known that certain species of bacteria are so small that they are near the limit of microscopic vision. Why may there not be other species that are still more minute, so minute in fact that they are beyond the resolving power of the best microscopes? I know of no good reason why ultramicroscopic bacteria should not exist. But after much work no one has been able to prove that such organisms do exist. Many hundreds of species of bacteria have been isolated and grown on culture mediums. Such isolations depend in no way on the size of the organisms.

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isolated. But all of the species that have yet been cultivated come within the range of our microscopes. This suggests that there may be a very definite lower limit to the size of living cells and that this limit may be near the limits of microscopic vision. Perhaps life refuses to be crowded into cells that are much smaller than 150 millimicrons in diameter.

It would, nevertheless, be very desirable if we could go beyond this limit in microscopic studies using visible light. But before this can be done it will be necessary to find new lens making substances and new immersion fluids. In the diamond and in zirconium and titanium oxides we have suitable lens making materials that would give higher resolution than any of the glass or quartz lenses now in use. A suitable microscope using diamond lenses would make visible objects about half the size of the smallest now seen under the best glass lens microscopes. This microscope would be of no use, however, unless a suitable immersion fluid could be found. Such a fluid must have an index of refraction of about 2.4 and be otherwise fit for lens immersion. A fluid having these properties is not available at the present time. Lenses having indexes of refraction greater than glass cannot, therefore, be used. It is evident that the first difficulty to be overcome is to find a new immersion fluid. One of the best immersion fluids now in use is monobromnaphthalene. This has a refractive index of about 1.66 and is suitable for use with lenses made of flint glass. If proper immersion fluids could be found there would be no serious difficulty in the way of making lenses from diamonds or other suitable materials except the expense of manufacture. Until better instruments are available we

must make the best possible use of the apparatus which we now possess. A microscope having quartz lenses and suitable for use with ultraviolet light has a resolving power approximately equal to that which one having diamond lenses would give. This apparatus is available and is being used in the study of viruses. But, since the eye cannot make use of ultraviolet light, objects studied under the quartz lens microscope are revealed only by means of the photographic plate. This is a great disadvantage because the making of such photographs is slow and difficult.

The ultramicroscope reveals objects that are very much smaller than those seen under the ordinary microscope, but it shows them only as points of light. The form of such objects is not revealed. The ultramicroscope has not proved the existence of ultramicroscopic organisms. A microscope equipped with diamond lenses and a proper immersion fluid would enable us to see objects that are about 75 millimicrons in diameter. Such an instrument might make possible the discovery of the so-called ultramicroscopic organisms that are thought to cause the virus diseases of plants and animals.

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ECOLOGY AND HUMAN AFFAIRS*

HENRY CHANDLER COWLES

In the early days of botanical development, the practical side of the subject was brought strongly into view. Indeed when botany began its modern development during the sixteenth century, the leaders in the subject were almost all men of medicine, who took to botany in order to find out the curative values of plants. Botany and medicine, for a century or more, were hand in hand, and progressed together. We owe to these men of medicine the foundation of systematic botany. It was not until the eighteenth century that systematic botany came fully into its own through the work of Linnæus. From that time forward the relation of botany to medicine was much less close than it had been previously, and the practical aspects of the subject were placed more and more in the background. This was increasingly true in the nineteenth century during which period various phases of botany came successively into being, namely, morphology, physiology, pathology, ecology, and genetics. In most of these phases practical applications were mainly incidental.

* Lecture given before the State University of Iowa, Iowa City, January 31, 1927, Academy of Medicine, Des Moines, Iowa, February 1; Iowa State College, Ames, Iowa, February 2, The Mayo Foundation, Rochester, Minnesota, February 3, and University of Minnesota, Minneapolis, February 4.

and little emphasized. It was necessary then to build the foundations of these various aspects of botanical science and there was little time or incentive left to work for practical applications. In the twentieth century, however, there has been an increasing development of botany along practical lines. In the eighteenth and nineteenth centuries botany and agriculture had little in common, but now they are recognized as kindred and related sciences, and it is becoming increasingly clear that botany is fundamental to agriculture and also that agriculture furnishes much of the best material for students of botany. The practical aspects of the subject are still much stronger in some phases of botany than in others. It is still not very evident in systematic botany and morphology, but it is increasingly prominent in physiology, ecology, pathology, and genetics. My purpose here is to indicate some of the intimate relations that exist between ecology and practical affairs.

Plant ecology may be defined as that phase of botany which endeavors to stress the relationship between plants and their environment. It can readily be seen, therefore, from the very nature of the definition, that ecology fundamentally is practical. Man is overwhelmingly dependent on plant life for his food, clothing, and many other necessities of life. Even where plants are not used directly they none the less cater indirectly to man's comfort. For example, animals which also supply food and clothing, themselves depend on plant life for their existence. The coal and oil, which are so important in industrial life, are due to the plant life of former ages. It is clear, therefore, that human industry and human life

itself would be quite impossible were it not for plants. It is fundamental in agriculture to know where plants grow best and why. The farmer must know the relation of his crops to temperature, light, water, oxygen, carbon dioxide, and mineral salts. All of these things are part of the crop environment. Plant agriculture therefore may be defined as practical plant ecology. Everything having to do with the cultivation of plants in the field and garden stresses the ecologic point of view. The modern science of forestry is also a division of applied ecology. In former times, when we depended entirely on native trees for lumber and fuel, it was not of prime importance perhaps to know the conditions of life for trees, but now that we have reached nearly the end of our natural lumber supply and must depend on reforestation, it is obvious that we must know where trees grow best and why. In the days to come we must treat trees as crops and know exactly their relations to their environment.

The general situation having been outlined in the preceding paragraphs, certain specific illustrations may be given of the fundamental importance of ecology in human life. One of the most interesting relations in the plant world is the relationship between plants and insects. Such insects as bees and moths play a fundamental part in seed production, by reason of the fact that their visits to the flowers occasion the transfer of the male elements to the female, and therefore make possible the growth of seeds. This important function, which is known as pollination because it involves the transfer of pollen, representing the male element, to the stigma, representing the female element, is due largely to the work of

insects. The insects, of course, visit flowers to secure nectar or in some cases pollen. In so doing they bring pollen from flowers previously visited and thus secure not only the transfer of pollen to the stigma, but also the crossing of one plant with another, a function which is believed to be very beneficial in the plant world. In those crops in which seeds or fruits are used, as in the cereals and the various fruits, pollination is in most cases necessary to secure the full development of the seeds and fruits. In corn and the other cereals the transfer of pollen is brought about by wind, but in most of the fruits the transfer of pollen is brought about by insects, as in the apple, plum, cherry, strawberry, and so forth. In nearly all the fruits flowers that are not visited by insects fail not only to produce seeds, but fail also to produce fruits. In some cases, as in the banana and the fig, pollination is not necessary, but in the apple, pear, plum, cherry, strawberry, cranberry and so forth the failure of pollination means a failure of fruit production. Not only this, but in most of these fruits it is necessary that the pollen come from other plants. One of the most interesting of all cases and one which has been known for only a few years is that of the apple. Most apples, such as the Jonathan, Winesap, and Delicious, must be pollinated by some other kind of apple. The Jonathan, for example, is sterile to its own pollen. To get a Jonathan apple we must have pollen brought from some other kind of tree. This, of course, means that a Jonathan apple has seeds of hybrid nature and this explains why it is that apples ordinarily do not come true to seed. All of this interesting matter has been known for only a short time. It was

first ascertained in Oregon through observation of serious failures in the apple crop under certain conditions. When the subject was analyzed closely, it was found that the apple growers had large orchards of one kind of apple, so that pollination from other kinds was made difficult or impossible. It is now the custom, in large orchards, to interplant different varieties, so that pollen will be available for crossing, and it is also common practice to keep honey bees since honey bees are the main agents for pollen transfer in the apple. The discovery of the fact that most apples are self-sterile has resulted in an enormous saving of money to apple growers. What is true of apples is true also of pears and cherries, but not of all fruits. It is obvious, therefore, that the growers of fruits must know about the environment of their trees. They must know whether their fruits are self-sterile or not. If they are self-sterile they must know which varieties are most satisfactory for pollination purposes and what insects or other agents are necessary in order to effect pollination.

One of the most important phases of plant ecology has to do with the relation of plants to soil and climate. Ecologists have divided up the plants of the world into various climatic types and into various soil types. They have ascertained, through long years of arduous study, some of the reasons why plants are scattered where they are through the world. They have learned to know that certain kinds of plants indicate certain kinds of soils, and that certain kinds of plants indicate certain kinds of climates. If one should find, for example, in a sub-tropical region the absence of all plants that are unable to withstand frost, it would be unwise to plant in that region

tropical plants that are not hardy. Certain natural plants are found to be characteristic of acid soils and certain others of alkali soils. Such plants therefore should give the farmer some indication of what he can safely plant. Where one finds blueberries and huckleberries growing the soil is acid and it would be unwise to plant such a crop as alfalfa in such a soil, since alfalfa is not tolerant of acid. One could, however, plant certain acid tolerant berries with prospect of success. Similarly, certain native plants indicate alkali soils, such as greasewood in the arid regions of the west. In greasewood soil it would not be wise to plant the ordinary sorts of crops. Some plants, such as sugar beets, are tolerant of quite a bit of alkali. Obviously farmers should know something of the native vegetation of the region where they cultivate their crops, since the native plants give precise indications of soil character and furnish a good clue to the types of crop best suited to such lands. Some striking cases illustrating these points may now be mentioned. Many years ago Dr. Macoun, the famous Canadian botanist, discovered growing in the Peace River District of Alberta many plants of widely southern range such as cacti. He was greatly surprised to find the cactus so far north. He concluded from its presence there that the climate must be fairly genial and suitable to wheat. At that time he was laughed to scorn, but the Peace River District is now recognized as one of the best wheat areas in the world. In Colorado it is safe to plant ordinary northern crops in regions inhabited naturally by the western yellow pine. This tree usually grows considerably below 10,000 feet in altitude, and most crops are cultivated in these altitudes. In one valley

in southern Colorado the pine grows at a much higher altitude than elsewhere and in this place crops can also be grown at a high altitude

One of the most important phases of plant ecology is the study of the succession of vegetation types. It has been discovered that plants succeed one another in definite stages. In the northern states, for example, the first trees to occupy a new area are such light tolerant trees as pines. Such trees commonly are succeeded by oaks, and oaks in turn by beech and maple. There is a definite succession from the most light tolerant to the most shade tolerant trees. Parallel with this change in the light quality within the forest is a change in the soil character. The first trees grow in soil that is relatively free from humus and comparatively dry. In succeeding stages there is an increase in humus and soil moisture. The final forest that occurs in the soil which is richest in humus and moisture and also in shade is known as the climax forest. A succession of vegetation from the pioneer conditions through to the climax forest is called a progressive succession. Under some conditions, mostly through human influence directly or indirectly, the succession may go backward. An area that is cut over or burned may revert to more primitive types. Such successions are known as retrogressive. One of the best studies of retrogressive succession in relation to matters of human concern has been made by Sampson in overgrazed lands in the west. In the western range lands, as in Utah, there is a definite succession of vegetation from weedy plants characteristic of dry soil through to a climax vegetation of nutritious grasses. When these grasses are overgrazed open

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places are made in the grassland and the seeds of weedy plants enter in, whereas the grass plants being eaten are not able to develop seeds of their own kind and carry on their types of vegetation. There results, therefore, a replacement of the climax grassland by relatively pioneer weedy growths that are unsuited for grazing purposes. Overgrazing therefore results in deterioration of pasture land and range land. Those who have charge of grazing animals should watch their areas closely and when they see evidences of retrogression they should defer the grazing period until after plants have ripened their seeds or should perhaps exempt the region from grazing altogether for a season or two until the land is able to recover. This whole question of the ecology of grazing lands is of enormous significance in furthering the meat supply of our country. If overgrazing is not checked, the situation goes from bad to worse and too frequently lands become not only reduced to a pioneer vegetation condition, but sometimes gullying sets in, hillsides are eroded, and the land becomes completely ruined for any advantageous purpose.

Another important relation of plant succession to human life is seen in connection with the work of the United States Land Office. In many instances original surveys of land have been shown to be inaccurate. Areas have been homesteaded and settled while other areas have been exempt from settlement because the land was classified incorrectly. For example, many areas in Arkansas were entered on the original surveys as lakes. These lands are not now lakes, but are covered with a heavy growth of timber. Ecologists have been called in to determine from the aspect of the present

growth what the facts probably were at the time of the survey. If trees one hundred years old are found in an area surveyed and regarded as lake land seventy-five years ago, it is obvious that the survey was incorrect. If the trees that are one hundred years old are upland trees rather than swamp trees, it is obvious that the mistake was even greater. For example, it would take many hundreds of years for vegetation to fill up a lake. It would take other hundreds of years for such an area to undergo a succession from the first swamp forms through the different stages to the eventual upland forest. A one hundred year old upland tree on such an area would mean that it could not have been a lake perhaps for one thousand years. All such problems enter unmistakably into the realm of plant ecology, and the ecologist, better than anyone else, is able to look at a tree and picture the conditions of the land on which it grows not only in its own lifetime, but in the lifetime of generations of trees now gone.

The foregoing illustrations of the application of ecology to human affairs could be multiplied indefinitely. When ecology started its great period of development about 1895, none of its devotees thought of it as of any great practical value. They were interested in the subject for its own sake without regard to human applications. It now becomes evident that ecology is one of the most practical of all subjects in plant science. Its bearings are many and intricate, and it may be confidently predicted that with future development the relationship of ecology to human life will appear still more evident and involved and will receive in this connection increasing recognition.

SOME ASPECTS OF THE FUSARIUM PROBLEM*

GEORGE HERBERT COONS

The development of plant pathology in the last three decades may be attributed to the building up of a strong body of facts concerning the causation of the diseases affecting our agricultural crops. The knowledge gained from etiologic studies has led to a rationality in control measures and has opened up methods of approach to the more intricate problems of plant pathology, the reactions of plants in disease. Another line of endeavor has been fruitful of result, namely, the development of strains of plants resistant to disease of one type or another, and to studies along this line we must hopefully look for amelioration of the disease situation which, with the ever increasing intensification of crops, presses upon us.

I am to discuss some aspects of the *Fusarium* problem to-night, and I shall consider first the extremely complicated situation which now exists because of the difficulty of classifying members of this genus of fungi and then will discuss the work which has been done in developing *Fusarium*-resistant plants.

In the beginnings of our science, the study of the causation

* Lecture given before the State University of Iowa, Iowa City, February 23, 1927, University of Minnesota, Minneapolis, February 24, and The Mayo Foundation, Rochester, Minnesota, February 25

of plant disease was apparently a simple matter. One had merely to swear allegiance to the Rules of Koch and turn to the handmaiden sciences, mycology and bacteriology, for methods and a solution. As a result of further research, the apparently simple etiologic situations have been found in reality extremely complicated. As precision of diagnosis increased, the large catch-all groups of organisms have been subdivided and subjected to drastic reclassification. Certain large groups of fungi, which by their pleomorphism had almost defied classification under the standard methods of the mycologist, still remain to vex us. The genus *Fusarium* has been noteworthy among these groups as presenting difficulties of classification for the taxonomist and of species diagnosis by the worker. The obstacles in the way of determination of the species of these fungi have hampered investigation in this line more than one who has not worked in the group can easily recognize.

The problems involved in the study of parasitic fungi do not stop with the species. Within a species, a more or less definite morphologic unit with more or less recognizable limits, there have now been found entities differing among themselves. In short, in the same fashion as the host plants exist in races, strains, and varieties, which differ from one another in all the ways which nature makes plants to vary, so too the fungous and bacterial parasites vary. Among the parasitic fungi we now recognize the existence of physiologic races within the species limits. These physiologic races of parasitic organisms are a present problem in plant pathology, a problem involving the study, charting, and diagnosis of these

new entities. And the problem becomes even more urgent when in our attempt to breed resistant plants we find not only must we consider breeding for a particular pathogen of a locality, but we must take into account the whole gamut of parasitic entities, classifiable, to be sure, under a single species, but as different in potentialities for the production of disease as can be imagined

As will become evident as this discussion proceeds, in studies in the genus *Fusarium* we meet both the problems of species determination and that of physiologic race differentiation, the latter a problem as yet but dimly outlined

The old mycologist, Link, erected the genus *Fusarium* to include the *Fungi Imperfecti* having hyaline mycelium and fusiform spores. Since that time the description of species has gone on, for the most part inadequately, until the old literature in this group presents an almost undecipherable confusion

The plant pathologists, too, have added their quota and hundreds of diseases are attributed to this genus. The early studies of *Fusarium* diseases in many cases are conspicuous by the paucity of detail in describing the causal organism. When summary was made of our progress it was found that the plant diseases attributed to this fungus were among the most serious known and they frequently were characterized by the fact that the capacity of the fungus for persistence in the soil rendered infested soils permanently unfit for the culture of the susceptible crop

The diseases associated with *Fusaria* may be put into a few general categories, groups, however, which do not have hard

and fast lines. We find that these organisms are responsible for seedling blights, rots, and wilts of plants. The list of hosts attacked by one species or another of this genus is long and our knowledge of the true extent of this group of fungi in its parasitic relations is as yet far from complete. Attention of plant pathologists has hardly been directed to the *Fusaria* associated as septic factors in the decay of plant parts or to seedling blights produced by this genus. The bulk of the research has dealt with the wilting diseases produced by *Fusaria* when the vascular systems of plants are invaded

It may be instructive to pass in brief review a few of the important diseases definitely to be attributed to members of this genus, in order to bring out the features in the attack of these parasites and to give some notion of the injury they are capable of causing. The host plants are to be found in every group of economic plants and the pathogens cause seedling and fruit blights in forest and fruit trees, seedling blights, grain molds, root and stalk rots of cereals, root rots and wilts of the fiber crops, root rots, wilts, and blights of one kind or another on vegetable and ornamental plants. Actually a listing of the susceptible plants from which *Fusaria* have been more or less adequately reported would be practically a listing of all economic hosts, so widespread are the fungi of this genus, so versatile the attack, and so important are they as pathogens.

The study of this disease complex with modern methods was opened, as is the case for so many diseases of plants, by the researches of Dr Erwin F Smith⁴⁴ on the wilt diseases of cotton, watermelon, and cowpea published in 1899. Dr. Smith as-

signed the disease on all these hosts to an ascomycetous fungus, *Neocosmospora vasinfecta*, which he believed to be the sexual stage of the *Fusarium* associated in the case of each host with a typical wilt disease. Dr. Smith's conclusions as to the relationships of the ascomycetous form and the various *Fusaria* have been challenged by other workers (Butler, Higgins), but his infection experiments and clear exposition of the disease as a vascular mycosis produced by *Fusarium* focused attention of all workers on diseases of this type.

The most significant outcome of Dr. Smith's work was brought about by his continued interest in the problem. Through his efforts Dr. W. A. Orton was able to carry on his brilliant researches which led to the development of wilt-resistant plants for combating the diseases which had been the subject of Dr. Smith's inquiry. These will be referred to later.

Still another major contribution dates from this experimental work, namely, the concept of fungous infestation of agricultural soils and its dangers. Dr. Smith, in 1899, as a result of his studies, fully grasped the importance of this situation, and he called attention to the serious consequences of the establishment of parasites of this type in our soils.⁴³ His statements have seen ample fulfillment in the ever increasing losses arising from soil-inhabiting parasites.

We are thus led naturally to the work of Bolley^{5, 6} and his concepts of "wheat-sick" and "flax-sick" soils, ideas which each year are found to have wider and wider acceptance. Bolley noted the decreasing yields of wheat in the Northwest as wheat culture continued in the same fields, year after year, and he attributed the decline of yields from the bumper

crops of virgin soil to the low yields of older fields, not to soil depletion, but to soil infestation by parasitic fungi. These were, for the most part, weakly parasitic forms which by their attack in the seedling stage and on the roots and culms of wheat depressed yields by making stands poor, and the remaining plants sickly. *Fusarium* spp. were among the suspected parasites.

Bolley⁶ most nearly proved his thesis of "sick" soils by his observations and experiments with flax, published in 1901, and in a series of minor accounts extending over a ten-year period. In his early publications, this veteran pathologist attributed the destructive flax disease which was so extremely prevalent on lands devoted to flax culture to the wilt-producing fungus which he named *Fusarium lini*. Where flax is grown more than one year on the same soil the yields decrease (Barker), whence the term "flax-sick" soil. Barker in his résumé of the flax wilt situation pointed out that the production of oil flax in this country has steadily decreased. The average annual production of flax seed from 1902 to 1911 was 23,749,000 bushels and from 1912 to 1921 it was 13,668,000 bushels. Before 1911 the United States had turned from a flax exporting country to an importing one. The center of flax production has steadily moved westward to new lands. Driven by its enemies, flax has become a new-land crop.

The symptoms of flax wilt are typical of plants affected with *Fusarium* wilt disease. Plants are subject to attack in any stage of development but are most susceptible in the seedling stage. In this stage they may simply damp-off. When older plants are attacked the disease follows a varied

course depending on conditions. If the attack is rapid and severe, complete wilting and death result. In other cases, the plants may appear only stunted and unthrifty. It is characteristic of diseased plants to turn yellow and shed their leaves. One-sided involvements occur, with one-sided response to the effects of the fungous invasion. As in other diseases of this type, there are pronounced vascular reactions, usually blackening or browning of the tissue.

In 1904, Smith and Swingle published their studies on the Fusarium wilt of potato, a disease of great economic importance. This also is a disease characterized by effects which can be traced to an invaded vascular system. The work of these writers is significant for our discussion for they were among the first to describe a Fusarium (*Fusarium oxysporum*) adequately enough to enable recognition later. To the enormous difficulties met with in describing a fungus whose chief characteristic seemed to be its variability, these authors applied every resource of their bacteriologic and mycologic technic in the study of the organism. They were the first to recognize the significance of the striking color changes in mycelium and medium produced by Fusaria, a subject which Bessey, in a fundamental study, explained on the basis of the cultural conditions presented.

In 1910, Dr. Smith⁴⁵ described the wilt of banana, the so-called Panama disease, which next to sugar cane mosaic is doubtless the greatest enemy of tropical agriculture. We must turn to the important work of Brandes,⁷ part of whose investigation was done in collaboration with Dr. Smith's laboratory for the most complete account of the banana wilt.

We will choose from this study, not the details of the fungous attack, but rather the statements he makes, largely on the basis of first-hand observation, 'on the distribution and injuriousness of this disease in the tropics, for I want to impress the significance of the fact that in diseases of this type we are dealing with organisms capable of rendering soil permanently sick, and not with organisms sporadic in their outbreaks.

Banana wilt occurs in Central and South America, Mexico, the West Indies, Hawaii, the Philippines and probably in India, Java, and Australia. Thousands of acres of banana land have been abandoned due to the ravages of this disease in Central America. The entire export banana industry of the Dutch colony of Surinam was destroyed in a period of about six years. Brandes points out that the banana wilt is not only injurious to the fruit and destroys immature plants, but it affects the value of the land:

"Destruction of the immature plants is the most serious phase of the disease. The plants wilt and die in enormous numbers, before any fruit is produced. The plant falls prostrate in a short time, but the rootstock is not immediately killed and may send up fresh suckers. These will invariably become diseased also, and only rarely produce a marketable bunch. .

"The third type of loss, namely depreciation in value of land is due to the fact that the causal organism may remain long periods in the soil, and finally increase to such an extent that bananas can no longer be grown. Since in banana-growing countries the greatest profit is derived from these plants,

and sometimes there is absolutely no sale for other products, it is evident that the land is greatly reduced in value or made utterly worthless ”

Similar stories of heavy crop losses could be recited for tomatoes of southern United States, of cabbage in Wisconsin, Iowa, and Ohio, of celery in Michigan, Colorado, and eastern sections. The root rot of corn has recently been brought to the foreground by studies of workers in the Department of Agriculture and the relations to other cereal diseases pointed out. The diseases which I have given passing mention have all been fungous wilts, diseases in which the pathogen produces its effects by invasion and growth in the vascular portions of the host. But in no case is the attack of the parasite limited to the vascular tissues, for these fungi under appropriate conditions produce rotting, seedling blights, and other disease states which we have learned to associate with *Fusarium* attack. The wilting phase which has been stressed is merely the striking thing in their parasitism.

I shall pass over with mere reference the great mass of work which has been done with the *Fusaria* which are known chiefly as rot producers, and these are legion. The work of Carpenter,¹⁰ Link, and Goss²⁰ on the rots of the potato tuber should be mentioned in this connection. Thus one might wander far afield from one crop plant to another describing a complex of symptoms, alike in their major details, reciting a story of losses and reviewing interesting and productive research work. But we must pass on to a consideration of the parasite and work which is preliminary to any studies on the nature of the disease caused, work which, mayhap, give us

some light on that which still evades us, the nature of disease resistance. Preliminary to any attack on the principles of parasitism, there must be a clearing of the ground and an exact delineation of the problems. Workers must arrive at some concordance of opinion as to the organism involved in this or that disease complex. This has more in it than mere agreement as to a name for a causal organism since it involves a recognition, under the disease aspects of the true situation as regards the *Fusaria* involved. Each contribution on *Fusarium* brings to light the inadequacy of our knowledge of the etiologic factor involved in pathologic conditions which we at one time believed well on the way to solution. For example, Goss,²¹ working on the wilt disease of potato, which, for years, had been assigned to *Fusarium oxysporum*, found that the wilt disease in Nebraska is largely due to another species with quite different parasitic habits.

A reason for this constant overturning of our presumably settled convictions is to be found in the difficulties inherent in the determination of the species of *Fusaria*. As has been said, this fungous group seems to be characterized by its variability. Every laboratory has its isolations of *Fusaria*, all awaiting some specialist to determine them. The problem of physiologic forms within the species, once the species themselves are determined, remains as yet largely untouched and its relative importance with this group of fungi can only be surmised from the significance found in other groups.

The basis for classification of the *Fusaria* was laid in 1910 by Appel and Wollenweber in their important contribution, "Grundlagen einer Monographie der Gattung *Fusarium*

(Link)." These workers brought order out of the chaos of inadequate descriptions and inaccurate diagnoses. They were the first to show clearly that in spite of irregularities of spore forms, spore shapes, and spore sizes, and vagaries in their appearance in culture, it was possible to obtain enough constancy to permit a classification if a definite technic was utilized.

This paper made possible a classification of *Fusaria* because of one feature, the recognition that there is for each *Fusarium* as it is grown in pure culture a period of normality and a fungus to be recognized must be in this condition of "Normkultur." This feature which is basic in their work is also its weakness, for the criteria of the norm, itself indefinable and often attainable with great difficulty after months of culture, are variable in the hands of different workers. Appel and Wollenweber conceived that *Fusarium* cultures showed three phases of development, "Ankultur," "Normkultur," and "Abkultur," types characterized by the kinds of spores produced and the relative activity of the culture in the vegetative and fruiting phases. The "ankultur" was believed unsuitable for diagnostic purposes since in this stage of development the microconidia and mycelial growths predominate. In the stage of normkultur, which too has its subdivisions, young, high, and old, the fungus produces relatively a large mass of spores, prevailing macrospores, and at the time of "hochkultur" these spores may be conceived as representing the normal for the species. Spores taken from such a suitable culture were, by their morphology, believed to be decisive for classification purposes. They placed the species studied

into sections and these sections have in Wollenweber's treatment of the subject been named and more and more amplified, each section being built up around a type species.⁴⁹ Appel and Wollenweber used other characters in differentiating *Fusaria*, such as the color of spores in mass, the character of the mycelium, and the stroma, the nature of the spore heaps, the presence or absence of sclerotia, the nature and position of chlamydospores, all these and many other manifestations on certain culture media, mostly vegetable plugs or stems of plants. They outlined a technic whereby it was possible to describe a species of *Fusarium* so that it could be recognized by some one else expert in this technic

Then during the five or six years after the publication of this paper there began intensive studies on the etiology of *Fusarium* diseases of plants and an attempt to delineate the species. The contributions of Sherbakoff, Wollenweber, Lewis, Harter and Field, Carpenter,¹⁰ Link, and Pratt fall into this early group of papers in which the usability of the Appel and Wollenweber methods was given rigorous tests. The soundness of the method is evidenced by the fact that these workers were able to identify organisms by using it, and organisms described with attention to the characteristics necessary for definition under this code have been recognizable by workers applying the same technic.

Since that time hundreds of species have been proposed and for some of these there are varieties as well. There is still lacking a monograph of the genus, the nearest approach being Wollenweber's contributions

We may then take stock of our progress with this group of

fungi, confessed by its specialists as a most complex one. The fungous species comprising this genus, as already may be surmised from the discussion of a few fusarial diseases, are widely distributed and many of the species appear cosmopolitan. It is not uncommon to find the species known originally because of association with crops of the temperate zone, associated with diseases of an entirely different crop under far different climatic conditions, even tropical. These fungi are typically denizens of the soil and they tolerate a wide range of soil reactions. The host range of the species of *Fusarium* cannot even be approximated for a single species. Enough work has been done that we may confidently expect that the extreme selective parasitism shown by many other fungi will not be found operative for this group. Certain species have been shown to attack a considerable range of plants, but extensive cross inoculations have not been made. We are not surprised to find that *Fusarium oxysporum*, the cause of Irish potato wilt, for example, is also a parasite of the sweet potato, an entirely unrelated plant. Then with this fungous group we are dealing with organisms extremely responsive to growth conditions and the responses of the fungi under cultural conditions in the laboratory are seemingly so kaleidoscopic as to make diagnosis without careful study of each culture hopeless. It is the consensus of all work on *Fusarium*, that under standardized conditions, with fungi in comparable states of developments, a norm can be established. But each of the isolations made in the course of work with this group presents its own problem. All workers agree that a synthetic medium whereby a standardization of nutrient conditions could be

attained would be highly desirable, but such a medium is yet to be developed

Not to be overlooked is the variety or physiologic race problem. Nearly every species when studied intensively either by inoculation tests or morphologic studies, has yielded a quota of variant forms whose classification seems to demand the erection of new varieties within the species, all to the discomfort of the general worker. In many instances the erection of these varieties serves a useful purpose. Beach, for example, working with the *Fusarium* wilt of aster, found he was dealing with a causal organism which differed in few morphologic and cultural characters from the cabbage wilt organism. But strong differences in pathogenicity were manifested by the aster wilt fungus and the cabbage wilt fungus (*Fusarium conglutinans*). Each produced a typical disease on its respective host, and the cabbage wilt organism was pathogenic to China aster, but the affected plants did not blacken as is characteristic in true aster wilt. The aster wilt fungus was not pathogenic to cabbage at all. Beach met the difficulty by calling the aster wilt fungus, the variety *callistephi* of *Fusarium conglutinans*. We do not have as yet a basis for interpreting the significance of such findings, but these experiments and many others of like tenor are the basis for the references I have made to the likelihood of the existence of physiologic specialization in this group.

The *Fusaria* associated with the potato plant in disease have been most fully charted of any. Appel and Wollenweber in their work dealt with thirteen species mostly potato. Sherbakoff in his memoir, "*Fusaria* of potatoes," listed the

twenty species previously described and forty-one new species and varieties. Morris and Nutting turned to these published accounts and using single spore cultures isolated from rotting potato tubers in Montana, attempted to diagnose a series of 100 cultures. They arrived after some years of work at a more or less definite diagnosis of ninety-seven of the cultures, some of their determinations being provisional and others made with reservations. They noted discrepancies in the descriptions. The organisms dealt with were put in seventeen of the described species. Their comments are pertinent to the present situation of *Fusarium* studies.

"The greatest obstacle in the way of accurate determination of species of *Fusarium* is the lack of a good monograph of the genus, and this lack is due in part to the nonstandardization of the methods used in identification work, especially as regards kinds of media, environmental conditions, and the relative value ascribed to various characteristics of the fungus when grown in pure culture under laboratory conditions. The species and varieties intergrade and the differential characters used in the keys are not sufficiently distinct to permit any but an experienced investigator to use the key. To become an authority one must work long enough and with large enough numbers of species so that he can create within himself a concept of the species. In other words, he judges to what species the fungus in question belongs rather than actually identifying it."

A second worker published his conclusions after attempting work on this complicated genus. Hansford, working with *Fusaria* isolated from suspected banana wilt material,

criticized the minute limits set up by the specialists for the wilt-producing species of *Fusarium* belonging to the section *Elegans*. He has dealt with 315 isolations from soils, from diseased banana plants, and from débris from banana plants. The species encountered in this study number thirty-five, but the greatest attention was placed on the *Fusaria* of the *Elegans* group. Hansford was able, using the accepted technic, to divide the organisms isolated from the vascular system of diseased bananas into five morphologically distinct divisions, and representatives of these when inoculated into banana plants produced the banana wilt disease. From soils he also obtained organisms which he could place into groups paralleling the pathogenic forms, but representatives of these forms did not prove to be pathogenic to banana. He stated: "From the present work it becomes evident that the only possible test for this organism (banana wilt) is that of inoculation into healthy banana plants. In other words *Fusarium cubense* is a purely biologic species as distinct from the purely morphologic species such as *Fusarium oxysporum* and so forth, and cannot be diagnosed from its morphologic characters as shown in pure culture.

"During the course of the present work the writer has been struck with the defects of the present system of classification of this genus, as given by previous workers. Up to the present it seems that the conception of a single species in this group of fungi has been kept too rigid, and does not allow a sufficient range of variability within the species. Especially is this the case in the sections *Elegans* and *Martiella*. The organisms

of the former include many parasites of crop plants and the workers on this group have endeavored by a minutely detailed examination of the characters of these fungi grown in culture under as far as possible closely specified conditions to separate these organisms from each other on morphologic characters, which necessarily show extremely minute differences, if indeed, any at all, between the various strains of the group. Not only are these differences between the so-called 'species' so minute, but at the same time each organism shows such a great range of variability in its morphologic characters that the differences used to separate the various 'species' are almost negligible by comparison. It appears to me that much would be gained and much labor spared, were the various sections of the genus which have been erected by Wollenweber and his colleagues to be considered as species rather than sections of the genus, and then the various species at present included in these sections could be regarded as forms of these large species which show a slight variation from a central type "

Whether the suggestions of Hansford would simplify the complex problem I cannot say, but I cite Hansford's experience as evidence of the baffling conditions that meet the worker in this field. We have demanded precision in diagnosis and now that we have it, its very complexity overwhelms us.

Recently the results of a conference of the leading specialists on the taxonomy of *Fusarium* have been published. (Wollenweber, Sherbakoff, Reinking, Johann, and Bailey.) In this article there is recognition of the variations in methods em-

ployed in the different laboratories and an attempt made to standardize procedure. The difficulties involved with this genus are admitted and recommendations are made which should be helpful. The methods outlined do not differ essentially from those already in vogue in these studies. Distinct advantages will arise from the descriptions (in the form of a key) of the fifteen sections now recognized within the genus.

Such, then, is the *Fusarium* situation at the present time. Workers in plant pathology are confronted with grave problems in the diagnosis, separation, and control of *Fusarium* diseases; the enormous accumulation of species, and the elaborate technic necessary in making determination of species have brought us to an impasse. I have no desire to disparage in any way the value of the taxonomic work which has been done on this genus. It has been of such a nature that we are forced to admire it. But I think we must frankly face the fact that the whole *Fusarium* problem is held up until methods can be devised which will help the worker, who is engaged in the study of *Fusarium* as a pathogen, to make dependable diagnoses. This isolated worker needs some ready method of identifying an organism and of separating in his work related forms which, although interesting of themselves taxonomically, are a hindrance in the solution of a problem in which a specific pathogen is involved. To those whose work leads them to apparently new forms, the question arises whether it is better to master a difficult technic and attempt to describe the fungus or to name it provisionally a form of some old species, thus confusing the situation rather than helping it.

It is not exaggerating the situation to say that probably only a half dozen people in the world at present are qualified to determine species of *Fusarium* and it is obvious that these workers, many of them not devoting their full time to studies of this sort, cannot be called on for solution of the problems of identification which arise so often in the laboratory. Every laboratory has its stock of undetermined *Fusaria* and any one bold enough to offer to diagnose *Fusaria* would be flooded

We have reached a point where progress in studies of plant pathology, in which this group is involved, hinges on the development of methods which give some promise of relief. I wish to present two small contributions to this phase of the subject which, while far from meeting the situation, at least have some promise of workability within the limits for which they are suitable of application. These methods which I wish briefly to detail are the product of experimentation carried on in my laboratory in collaboration with Miss Miriam Carpenter.

In our first method we sought to apply serologic tests to this problem. In spite of the conspicuous success of serologic methods of diagnosis in the field of animal pathology, the literature of plant pathology contains but few reports of utilization of these methods in the diagnosis of bacterial and fungous pathogens. Fred and his associates^{18, 47} applied serologic technic in their studies of *Bacillus radicum*. Brooks and his associates used this method with a few bacterial pathogens and Riker reported immunization of rabbits with the crown gall organism, and the utilization of the sera with

suspected cultures. Goldsworthy utilized a high-titer anti-serum to identify *Bacterium maculicolum* suspects plated from soils.

It is evident that for the bacterial pathogens, agglutination tests offer wide possibilities for quick determination of suspects and for deciding relationships, subject of course to the limitations of the method

For the fungi, if we exclude the fungi pathogenic to animals, we find only casual attempts to use serologic reactions. The conspicuous advance made by Mez and his students in the utilization of precipitation and conglutination reactions as an index to plant relationships has now become fairly well known in the United States and we may expect increased interest in the subject. This work has been largely confined to the higher plants, or at least to those forms which yield sufficient quantity of material to make the tests feasible. Work of this sort seems to stand on safe ground, as evidenced by the classic work of Wells and Osborne on the specificity of serum reactions when pure plant proteins were used as antigens. The interesting relationships brought out by serum reactions are in the future to be more and more taken into account in phylogenetic studies

Application of serologic technic to fungous studies were made by Schütze⁴⁰ in 1902, in his precipitin tests to determine the relations of various types of yeasts. He compared the reactions obtained with sera from animals sensitized with upper and lower brewery yeasts and with the yeasts from bread and potato, and he stated that even with the application of the "most beautiful and most sure biologic" technic

often repeated, he could not obtain differences. In later work,⁴¹ using the complement fixation reaction, this worker was able to separate the yeasts of the upper and lower fermentation, but he was not able to differentiate the bread and potato yeasts by this test. Citron showed that the fungi causing favus formed a similar precipitin since a serum produced by infections of *Trichophyton microsporon* gave precipitation with the *Trichophyton* from cats and extracts from favus material from mice and from human beings. Magnus and Friedenthal in order to test the genetic relationship of the higher fungi, yeasts and the truffle, *Tuber brumale*, injected animals with expressed sap with the following results: Although the reaction with yeast, truffle, and *Agaricus campestris* is throughout specific, and although the serum from an animal immunized with truffle material gives a precipitate only with its homologous antigen, the serum of the animal sensitized with the yeast antigen gives a precipitate with the juice from truffle as well, and therefore, they concluded, the yeasts are more closely related to the truffle than to the higher fungi.

In the early work with fungi as antigens, I tested the sensitization of guinea pigs by observing if anaphylaxis occurred with subsequent delayed injections of the homologous antigen in comparison with the results of crossed injections with a heterologous antigen. Guinea pigs when injected with material from fungus cultures were sensitized by these injections as evidenced by their behavior and in most cases death on the second injection with the homologous antigen. Guinea pigs

sensitized with small injections of *Phoma* sp. were killed by a second injection of this material, while companion animals similarly sensitized tolerated injections of different species only to be killed by injections of the homologous antigen on the succeeding day. We carried on enough of these trials to convince us that specific sensitization was being built up by the extremely small amounts of protein we were able to inject using fungous mycelium, but gave up the method as not being usable for our purpose. We were not able to secure results using the elevations of temperature as an index of anaphylactic reaction as has been done by some workers.

We then cast about for other methods which could be made to serve. Preliminary work with the precipitin test was not promising since we were not able to produce a high-titer serum because of the scantiness of our injection material and the comparatively high dilution of our antigens. For it must be remarked that this factor, the obtaining of sufficient suitable antigen and its standardization, is the great obstacle in experimental work of this type.

We then turned to the complement fixation test as a method fine enough to detect the very small amount of reacting substance possible of production considering the nature of our material. The methods employed in our work have been dictated by the type of growth with which we have had to deal. The members of this genus produce an abundant growth on all laboratory media, but in the aggregate this represents only a small dry weight of substance, especially since we have wished to work with young cultures (ten-day). The fungous mats which are obtainable after growth on a liquid culture

medium (we have always used a synthetic medium* with potassium nitrate as a source of nitrogen) consist of cottony wefts of mycelium with more or less spore material included. The walls of the fungus are extremely refractory to dissolution, and the protein content is locked up within these walls so that extraction is extremely difficult. Antigens have commonly been prepared by drying the mycelial mats and grinding them in a mortar with an aliquot amount of salt so that on subsequent dilution the material was suspended in a physiologic sodium chlorid solution. This method is tedious and fraught with the dangers associated with large particles which escape grinding. It has been found possible to sensitize rabbits with antigens prepared in this manner.

The usual methods employed have consisted of a series of intravenous injections at two-day intervals and, in general, five injections of 5 c. c. are given. The antigens were made as has been indicated from washed mycelial mats grown on synthetic solution. The dried antigen was weighed and ground to fineness in a mortar with salt. At the time of use water was added so that 0.1 gm. of mycelium was used in 5 c. c. of distilled water. After about ten days the animals are then bled from the heart, and the serum obtained, inactivated, and preserved in the usual manner. Sera thus obtained have been tested against the homologous antigen and against some

* The synthetic medium used throughout these tests and in the serologic tests is one devised so as to give a strong mycelial growth and contains Saccharose, 7.2 gm., dextrose, 3.6 gm., magnesium sulphate, 1.23 gm., potassium acid phosphate, 2.72 gm., potassium nitrate, 2.02 gm., water, 1000 ml., and agar agar, 12 gm., added to make a solid medium.

related forms. A typical test with sera prepared in this manner is shown in Table 1.

Rabbit 301 was sensitized with *Fusarium radiculicola* and Rabbit 303 was sensitized with *Fusarium martii phaseoli*. Sera from these animals diluted 100 times were tested against

TABLE 1
COMPLEMENT FIXATION TESTS WITH FUSARIA
Reaction Against a Homologous Antigen Compared with the Reaction Against Heterologous Antigens

Schedule	Test						Check	
Complement	0 1	0 1	0 1	0 1	0 1	0 1	0 1	
Serum 1 100	0 05	0 04	0 03	0 02	0 05		0.05	0 05
Antigen 1 200*	0 05	0 05	0 05	0 05	0 03	0 05		0 05
Results								
Serum 301 with F 313 (homologous)	+	+	+	=	-	-	-	+
Serum 301 with F 156	-	-	-	-	-	-	-	+
Serum 301 with F 348	-	-	-	-	-	-	-	+
Serum 303 with F 348 (homologous).	+	+	+	+	+	-	-	+
Serum 303 with F. 156	=	-	-	-	-	-	-	+
Serum 303 with F. 313	-	-	-	-	-	-	-	+

* For tests, supernatant liquid over the ground mycelium which had been used for sensitizing animals, was used.

F. 313, *Fusarium radiculicola*.

F. 156, *Fusarium conglutinans*.

F. 348, *Fusarium martii phaseoli*.

their own antigens and cross tested. They were also tested against *Fusarium conglutinans*. For antigens in the fixation test, the supernatant liquid of the material such as was used for injection purposes was diluted 200 times in physiologic sodium chlorid solution. It should be mentioned that preliminary to injection, each rabbit was tested for natural fixing bodies and was found negative. So far it has not been necessary to reject any rabbits on this score. Tests to determine the anticomplementary dose for both the sera and the antigen were made at each test and quantities chosen well below the limits. In this test it will be noted that the sensitized sera have fixed 2 units of complement when in the presence of the homologous antigen and have been effective in fairly low dilution.

An important and, for a time, vexing factor arose in our tests, and that is the variability of complements. We lost much time and had many tests which were not consistent which we now attribute to the variation in the fixability of the various complements. Bearing in mind the picture shown by the last test, a comparison was made with the results as shown in Table 2. In this test the complement was used in quantities of 1.5 and 2 units. It must be noted that in these tests we are dealing with an antigen which does not produce any disease condition in the animal, and we probably cannot expect to find a large amount of antibody in the blood. It will be seen that complement B showed a far greater fixability than complement A and would permit of far more delicate differentiation. Neither of these complements was as suitable for the test as the complement used in the first test. We

TABLE 2
COMPLEMENT FIXATION TESTS WITH FUSARIA
Comparison of Reactions When Different Complements Were Used Animals Sensitized with Ground Mycelium

Schedule	Test															Check
Complement	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 08	0 1
Serum 1 100	0 1	0 08	0 06	0 05	0 05	0 02	0 1	0 06	0 04						0 1	0 1
Antigen 1 · 200	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05	0 05		0 05
Results	Complement A															
Serum 301 with F 313	+	+	±		±	±	±	-	-	-	-	-	-	-	-	+
Serum 301 with F 348	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Serum 303 with F. 348	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Serum 303 with F 313	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	Complement B															
Serum 301 with F 313	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+
Serum 301 with F 348	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Serum 303 with F 348	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+
Serum 303 with F 313	+	±	±	-	-	-	-	-	-	-	-	-	-	-	-	+

F 313, *Fusarium radicicola* F 348, *Fusarium marthii phaseoli*

found some animals which gave complements which did not show fixability although normal in their reactions in the hemolytic system. It is therefore necessary to test each complement separately, and to eliminate animals which give refractory complement.

We have also used a method developed in a study of the legume organism by Dr. R. M. Snyder of the Michigan State College, whereby he has been able to disperse dry plant tissue with selenium oxychlorid. Dr. Snyder has used peptized leaves of plants to incorporate in bacteriologic culture media and has some evidence that aside from the adsorbed selenium, proteins of the material are unchanged in the peptization process. We have applied Dr. Snyder's method to the dry mycelium and our work with the peptized mycelial mats of *Fusarium* seems to give strong corroborative evidence of the correctness of Dr. Snyder's interpretation.

We have found that animals tolerate this peptized material with its absorbed selenium if the material is well washed and the colloid resuspended after centrifugalization. The method of preparation of this antigen in a typical test was as follows: 0.87 gm of dry mycelium was dissolved in selenium oxychlorid and poured into 100 c c of distilled water, 15 c c of this solution was centrifugalized, washed six times, and then resuspended in 10 c.c of sodium chlorid solution. There was, therefore, in an injection quantity of 5 c c approximately 0.06 gm of dried mycelium given at each injection. Animals will tolerate at least 8 c c. of such a suspension, and probably much more, without ill-effects.

The results of a series of immunizations using the selenium

method of antigen preparation are given in Table 3 The antigen used was made from ground fresh mycelium extracted in sodium chlorid solution so as to give approximately a 1 : 20 dilution These tests, while not so decisive as other tests we have made with the ground type of antigen, show a strong general trend in favor of the homologous antigens, but there are some exceptions Certain animals are more suitable for building up antibodies than others I am mentioning this utilization of selenium oxychlorid because of its wide applicability.

Since we have been spending much time on this phase of the work, it is perhaps fitting to give our impressions of the possible utilization of the method in *Fusarium* research by phytopathologists We have been successful in demonstrating by means of the complement fixation reaction differences between closely related organisms and between a species and its variety. The reactions are extremely specific This test is very delicate and the many factors involved make it a test whose manipulation is not easy We believe it will be possible to develop sera of far greater potency than any we have so far obtained and sharper and sharper distinctions can be made As a tool for research where sharp differentiation of species and variety are required, we feel that the complement fixation test has great possibilities. For the general worker we feel that unless precipitation and simple agglutination methods can be devised, the serologic tests will not be of great service.

It cannot be gainsaid that there are now facing us enormous problems in relationship and, for the many reasons outlined,

TABLE 3

COMPLEMENT FIXATION TESTS WITH FUSARIA

Sera from Rabbits Sensitized by Use of Dried Mycelium Peptized with Selenium Oxychlorid and Tested Against Ordinary Ground Mycelium Extracts

Schedule		Test.				Check	
Complement		0 15	0 1	0 15	0 1	0 1	0 1
Serum 1	10	0 1	0 1	0 05	0 05	0 1	0 1
Antigen		0 05	0 05	0 05	0 05	0 05	0 05

Results							
Serum	Antigen						
199	<i>F. radicum</i> (Homologous)	±	+	-	±	-	+
	F of aster wilt (Heterologous)	-	-	-	-	-	+
	<i>F. maritima</i> I (Heterologous)			-	-	-	+
198	<i>F. radicum</i> (Homologous)	-	±	-	±	-	+
	<i>F. oxysporum</i> (Heterologous)	-	±	-	±	-	+
190	<i>F. maritima</i> I (Homologous)	-	+	-	+	-	+
	<i>F. oxysporum</i> (Heterologous)	-	-	-	-	-	+
191	<i>F. maritima</i> I (Homologous)	±	+	-	-	-	+
	<i>F. oxysporum</i> (Heterologous)	-	-	-	-	-	+
195	a-F of aster wilt (Homologous)	±	+	-	±	-	+
	b-F. of aster wilt (Homologous)	-	±	-	-	-	+

TABLE 3
COMPLEMENT FIXATION TESTS WITH FUSARIA.—*Continued*

Serum	Antigen							
195	a- <i>F oxysporum</i> (Heterologous)	-	±	-	±	-	-	+
	b- <i>F oxysporum</i> (Heterologous)	-	-	-	-	-	-	+
194	F aster wilt (Homologous)	-	+	-	-	-	-	+
	<i>F oxysporum</i> (Heterologous)	-	-	-	-	-	-	+
186	<i>F orthoceras</i> (Homologous)	+	+	+	+	-	-	+
	<i>F oxysporum</i> (Heterologous)	+	+	-	-	-	-	+

the morphologic approach cannot completely answer the questions which arise. For the solution of these problems in relationship we may confidently expect that the serologic methods will be of service

As our work progressed and we began to feel the limitations of the serologic methods, we have sought other biologic methods to help in the attack on this problem of differentiating pathogens. Our first attempts were concerned with a search for differentiating media. Research along this line was begun in 1922 by Miss Carpenter and such progress as has been made is due to her fidelity in the attack upon this problem. The early work represented a trial of a variety of synthetic media with and without the various indicators and chemicals utilized in bacteriologic research for similar purpose. Without going into detail as to these, suffice it to say that none

of the common indicators or none of the various carbon and nitrogen sources tried in connection with our base nutrient solution seemed to give great promise.

We were then led to the use of anilin dyes as toxic agents with the hope that the various species would exhibit different orders of tolerance to the toxic agents

The use of dyes in selective media has become a definite part of bacteriologic technic in the last decade. One has merely to refer to Endo's fuchsin-sulphite agar or to Holt-Harris and Teague's eosin-methylene-blue agar used for differentiating *Bacillus typhosus* colonies from *B. coli* as examples of this technic. Krumweide, Pratt, and McWilliams used two concentrations of brilliant green for typhoid differentiation, while in 1915 Petrof used gentian-violet as a differential dye in media for the isolation of the bacillus of tuberculosis, and this medium has found still wider uses.

The basic work along this line is found in the contributions of Churchman on the behavior of bacteria to gentian-violet. Churchman¹² demonstrated the parallelism between the Gram reaction and the gentian-violet reaction taking place on his plates and his observations have had wide application not only in cultural work, but in clinical medicine as well. Churchman¹³ has found that this selective bacteriostasis is not limited to gentian-violet, but is shown by other members of the triphenylmethane series.

The development of the work on fungi with the anilin dyes took the following course. In Miss Carpenter's thesis work,¹¹ twenty-one water-soluble dyes selected from the several classes of dyes were used:

Monazo	Chrysoidine Y, orange G, and Ponteau B
Diazo	Diamine blue, benzopurpurin, Congo red, Biebrich scarlet
Diphenylmethane	Auromine O
Triphenylmethane	Rosaniline, malachite green, isamine blue, crystal violet, brilliant green
Xanthane	Eosine (yellow), Rhodamine B
Acridine	Acridine yellow
Hydrazone	Tartrazine
Quinone-imide	Nile blue A, Magdala red, methylene blue (bacteriologic)

The results with these dyes in various concentrations when used in a synthetic medium* are given in Table 4, using three species of *Fusaria*. It will be noted from the table that the greatest checking of growth took place in malachite green, brilliant green, and crystal violet, dyes of the triphenylmethane series. In a series of tests using these dyes on synthetic media containing agar it was found that gentian-violet was less toxic than malachite green, and malachite green less toxic than brilliant green, so far as one can judge from percentage relationships.

The results of preliminary work with plate cultures of various species of *Fusarium* are shown in Tables 5 and 6, and these results form the basis of our later work. The malachite green was used in 1:40,000 concentration and the gentian-violet 1:26,000.

The presence or absence of growth is, of course, the most distinctive thing with any toxic medium. In the lower con-

*The synthetic medium used throughout these tests and in the serologic tests is one devised so as to give a strong mycelial growth and contains. Saccharose, 7.2 gm, dextrose, 3.6 gm, magnesium sulphate, 1.23 gm, potassium acid phosphate, 2.72 gm, potassium nitrate, 2.02 gm, water, 1000 ml, and agar agar, 12 gm, added to make a solid medium.

rations more or less growth takes place, and this has certain characteristics. Some species of *Fusaria* show a natural tendency to produce colored threads, and these colors may be blue, or brown, but the majority of the forms are white.

mycelium may grow entirely within the medium "submerged" or it may show considerable aerial mycelium. The amount of aerial mycelium under controlled environmental conditions seems constant for the species. The growth may be colony, showing mycelial threads intermingled to make a colony pad or felt. A colony with soft, short, hair-like growth has been termed "villous," while those mats showing long threads which have a tendency to clump together to distinct wisps have been termed "sericeous-tomentose." Marked differences occur with certain species in their effects on the substratum. Certain species, such as *F. radicicola*, dehydrate the medium so that not the slightest trace of color remains near the colony. Other organisms produce a range of color changes. Zonation frequently occurs in some species, but the great majority do not affect the color to any extent. The range of variation of the forms tested is great and it is in this difference in response we have a means of differentiating forms rather easily.

This paper is not the proper place to give details of the specifics of these methods nor an extensive review of our findings to date. Suffice it to say that we are trying to catalog all the different species we have been able to obtain as to their reactions on various concentrations of dyes.

We have grown many of these species several times and recorded their reactions. With extremely few exceptions, each

centrations more or less growth takes place, and this has certain characteristics. Some species of *Fusaria* show a natural tendency to produce colored threads, and these colors may be red, blue, or brown, but the majority of the forms are white. The mycelium may grow entirely within the medium "submerged" or it may show considerable aerial mycelium. The type of aerial mycelium under controlled environmental conditions seems constant for the species. The growth may be cottony, showing mycelial threads intermingled to make a cottony pad or felt. A colony with soft, short, hair-like growth has been termed "villous," while those mats showing long silky threads which have a tendency to clump together to form distinct wisps have been termed "sericeous-tomentose." Marked differences occur with certain species in their effects on the substratum. Certain species, such as *F. radicola*, decolorize the medium so that not the slightest trace of color remains near the colony. Other organisms produce a range of color changes. Zonation frequently occurs in some species, while the great majority do not affect the color to any extent.

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Rhodamine	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Eosine	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Acridine	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Tartrazine	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Nile blue	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Magdala red	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Methylene blue	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Checks	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-



TABLE 5

REACTIONS OF FUSARIA OBTAINED WITH A SYNTHETIC MEDIUM CONTAINING MALACHITE GREEN, 1 · 40,000, PERIOD TEN DAYS

Name of organism	Culture number	Size of colony			Type of colony		Type of mycelium		
		No growth	Diameter more than 2 cm	Diameter less than 2 cm.	Submerged	Center aërial.	Aërial mycelium abundant	Cottony	Villous
F orthoceras		-	-	+	+	-	-	-	-
F orthoceras var longius		-	-	+	-	-	+	+	-
F. conglutinans	156	-	+	-	-	-	+	+	-
F. conglutinans	165	-	+	-	-	-	+	+	-
F conglutinans var callistephi		-	+	-	-	+	-	+	-
F redolens		-	+	-	-	-	+	-	-
F sclerotoides		-	-	+	-	+	-	-	+
F oxysporum	209	-	+	-	+	-	-	-	-
F oxysporum	211	-	+	-	+	-	-	-	-
F oxysporum	204	-	+	-	-	-	+	-	+
F oxysporum	160	-	+	-	-	-	+	-	+
F. oxysporum	212	-	+	-	-	-	+	-	+
F. oxysporum	208	-	+	-	-	-	+	-	+
F euoxysporum		-	+	-	-	-	+	+	-
F vasinfectum		-	+	-	-	-	+	+	-
F. batatis		-	-	+	+	-	-	-	-
F. tricothecioides		+	-	-	-	-	-	-	-
F discolor		+	-	-	-	-	-	-	-
F discolor var sulph		-	+	-	-	+	-	-	+

TABLE 5—*Continued*

Name of organism	Culture number	Size of colony			Type of colony			Type of mycelium		
		No growth	Diameter more than 2 cm	Diameter less than 2 cm	Submerged	Center aërial	Aërial mycelium abundant	Cottony	Villous	Sericeous-tomentose
<i>F. culmorum</i>		+	—	—	—	—	—	—	—	—
<i>F. eumartii</i>	206	+	—	—	—	—	—	—	—	—
<i>F. eumartii</i>	171	+	—	—	—	—	—	—	—	—
<i>F. radicicola</i>	157	—	+	—	—	—	+	+	—	—
<i>F. radicicola</i>	202	—	+	—	—	—	+	+	—	—
<i>F. radicicola</i>	203	—	+	—	—	—	+	+	—	—
<i>F. radicicola</i>	207	—	+	—	—	—	+	+	—	—
<i>F. solani</i>		—	+	—	—	—	+	—	—	+
<i>F. coeruleum</i>		+	—	—	—	—	—	—	—	—
<i>F. zonatum</i>		—	+	—	—	—	+	+	—	—
<i>F. bulbigenum</i>		+	—	—	—	—	—	—	—	—
<i>F. aurantiacum</i>		—	+	—	—	—	+	+	—	—
<i>F. mali</i>		—	—	+	—	—	+	+	—	—
<i>F. asclerotium</i>	56	—	+	—	—	—	+	—	—	+
<i>F. asclerotium</i>	213	—	+	—	—	—	+	—	—	+
<i>F. asclerotium</i>	207	—	+	—	—	—	+	—	—	+
<i>F. lycopersici</i>	170	—	—	+	—	+	—	—	—	+
<i>F. lycopersici</i>	153	—	—	+	—	+	—	—	—	+

species has shown a distinct reaction to the toxic media. Age and past history of the culture do not seem to affect the response, since progenies of the same parent cultures received

TABLE 6

REACTIONS OF FUSARIA OBTAINED WITH A SYNTHETIC MEDIUM CONTAINING GENTIAN-VIOLET, 1 26,000, PERIOD TEN DAYS

Name of organism	Culture number	Size of colony		Type of colony		Type of mycelium				
		Diameter more than 2 cm	Diameter less than 2 cm	Submerged	Center aërial	Aërial mycelium abundant	Cottony	Villous	Sericeous-tomentose	Medium de-colored
F orthoceras		+	-	-	+	-	-	-	+	-
F orthoceras var longius		+	-	-	-	+	-	-	+	-
F. conglutinans	156	+	-	-	-	+	-	-	+	-
F conglutinans	165	+	-	-	-	+	-	-	+	-
F conglutinans var callistephi		+	-	-	-	+	-	-	+	-
F redolens		-	+	-	-	+	+	-	-	-
F. sclerotoides		+	-	-	-	-	-	-	+	+
F oxysporum	209	+	-	-	-	+	+	-	-	+
F oxysporum	211	+	-	-	-	+	+	-	-	+
F oxysporum	204	+	-	-	-	+	-	-	+	-
F oxysporum	160	+	-	-	-	+	-	-	+	-
F oxysporum	212	+	-	-	-	+	-	-	+	-
F oxysporum	208	+	-	-	-	+	-	-	+	-
F euoxysporum		+	-	-	-	+	+	-	-	-
F vasinfectum		+	-	-	-	+	+	-	-	-
F batatis		+	-	-	-	+	-	-	+	-
F tricothecioides		-	+	-	-	+	+	-	-	-
F discolor		+	-	-	-	+	-	-	+	-

TABLE 6.—*Continued*

Name of organism	Culture number	Size of colony		Type of colony			Type of mycelium				
		Diameter more than 2 cm	Diameter less than 2 cm	Submerged	Center aërial.	Aërial mycelium abundant.	Cottony	Villous	Sericeous-tomentose.	Medium de-colored	Deep purple zone below
<i>F. discolor</i> var. sulph		+	-	-	-	+	-	-	+	-	-
<i>F. culmorum</i>		+	-	-	-	+	+	-	-	-	-
<i>F. eumartii</i>	206	-	+	-	-	+	+	-	-	-	-
<i>F. eumartii</i>	171	-	+	-	-	+	+	-	-	-	-
<i>F. radicicola</i>	157	+	-	-	-	+	+	-	-	+	-
<i>F. radicicola</i>	202	+	-	-	-	+	+	-	-	-	-
<i>F. radicicola</i>	203	+	-	-	-	+	+	-	-	-	-
<i>F. radicicola</i>	207	+	-	-	-	+	+	-	-	+	-
<i>F. solani</i>		+	-	-	-	+	+	-	-	+	-
<i>F. coeruleum</i>		-	+	+	-	-	-	-	-	-	-
<i>F. zonatum</i>		+	-	+	+	-	+	-	-	-	-
<i>F. bulbigenum</i>		-	+	-	-	+	-	-	+	-	-
<i>F. aurantiacum</i>		+	-	-	+	+	+	-	-	+	-
<i>F. mali</i>		+	-	-	-	+	+	-	-	-	-
<i>F. asclerotium</i>	56	+	-	-	-	+	-	-	+	-	-
<i>F. asclerotium</i>	213	+	-	-	-	+	-	-	+	-	-
<i>F. asclerotium</i>	174	+	-	-	-	+	-	-	+	-	-
<i>F. lycopersici</i>	170	+	-	-	-	+	-	-	+	-	+

from different laboratories show identical reactions, and where they have been correctly determined show the characters of the species

We have not found any correlation of response to the findings of morphologic studies, that is, the reactions are individual species and variety reactions and not group reactions

We are greatly impressed by the possibilities of the method as a possible means of quick diagnosis and as a means of comparing a suspect with a known form, but it is as yet too early to indulge in speculation as to its general utility. As a laboratory method it would certainly be easy of utilization and prompt in its response. We would, however, advance the general thesis that the complicated *Fusarium* situation can best be helped by applying to the problem the best methods that have proved of service in other fields of research and this in a small way we have attempted.

Let us now pass to another phase of the *Fusarium* problem, a phase which has been conspicuous for its victories. Here we will find some noteworthy advances, not alone applicable to *Fusaria*, but to other pathogens as well

When Dr Smith had completed his studies on the pathology of cotton, watermelon, and cowpea wilt, he assigned to Dr W A Orton the problem of developing control measures for these diseases. Orton's brilliant researches,^{34, 35} over a period from 1899 to 1909, are classic in our science. Each disease problem was met by a separate solution. To combat the cowpea wilt, a variety was found which was also immune to nematode attack. To combat the cotton wilt, Orton did work which is today operative in all attempts to improve cotton varieties, namely, the selection, from among desirable strains of those plants which survive on *Fusarium* infested soil. Many of the strains of cotton now popular in the South trace

back to these original selections. The effect of the resistant strains was spectacular. Fields which were a failure when planted with commercial seed, grew profitable crops when planted with the new selections.

With the wilt disease of watermelon, the work done was remarkable considering the time at which it was conceived and carried out. This was in the period from 1900 to 1905 before Mendel's law had come to give new impetus to studies in genetics and when notions of hereditary entities were extremely vague. A basic thing in Orton's work was the development of a method of producing practically 100 per cent infection. Using manure infested with the causal organism, both from pure culture and from trash from diseased fields in the hills of melons, and planting, as well, on soil known to harbor the fungus, Orton was able to give all the available varieties of watermelon the severest of tests. He found none of them resistant. The citron, a melon-like plant used for stock feed, was resistant, and this when crossed with the watermelon gave a motley progeny. Selections made under conditions of heavy infection, in the second generation gave plants that were watermelons in type but with the added quality of resistance obtained from the resistant grandparent. These plants in repeated tests in succeeding years retained their qualities and the problem was solved apparently except, as Orton says, "the styles in watermelons changed." Market demand for a long melon of the Tom Watson type rendered the round type of melon, which Orton had produced, unwanted. But the contribution is there, and the method is an example and inspiration to future research.

We will now review the well-known work of Jones^{26, 27} and his students, Gilman, Walker, and Tisdale, in applying the selection method of Orton to the cabbage wilt situation in Wisconsin, work which has resulted in the rehabilitating of a moribund industry. Beginning in 1910, Jones, working with late cabbage of the Hollander or ball-head type, compared the commercial varieties as to relative resistance and sought to select outstanding individuals. Experiments were located in soil thoroughly "cabbage-sick" with *Fusarium*. Something of the situation can be gathered from the reports of their experiments when we read that, out of 5,500 plants set out, only eighteen were alive and only two of these headed at the end of the season. Selections made in 1910 from farmers' fields of plants which had produced heads under conditions of severest soil infestation were brought to seed production and tested on infested soil. Yearly reselections from this material gave the foundation for the seed stocks developed by the Wisconsin workers. Probably the best known of the progenies came from plants selected from the Broesch field, and on this half acre field only twelve plants headed, three of which were used for seed production. The results of the selections were immediately decisive in showing the value of the lines which had been started, for in 1912 in a field where commercial varieties were a practical failure, the seed from selected plants showed about 90 per cent of the plants living, and a high percentage of these produced heads. The Wisconsin workers state that the poorest among the selected head strains averaged better than the best of the parent strains, and they state that they found their greatest stimulus to continued selec-

tion in the significant differences in the behavior of the various head strains, some of which showed up far better than others. Tests in 1914 with second generation seed, selections again having been made, showed the marked superiority of the selected seed over commercial, viewed from the standpoint of percentage of disease, living plants, marketable heads produced, or tonnage. Without going into the mass of figures which so eloquently tell the story, the matter can be summed up from the data of the experiments, by stating that commercial seed used as checks produced 2 tons per acre, whereas the seed from plants selected from the Broesch field in their second generation produced never less than 8 tons and averaged 12.6 tons of marketable cabbage, a figure far above the ordinary for cabbage. Since 1915 the work with late cabbage has been continued and now Wisconsin Hollander is an established seed stock available for all who need it. The work has been extended with success to other types of cabbage.

In the control of flax wilt we find that Bolley^{5, 6} in his pioneer work obtained success in development of wilt-resistant flax by both mass and individual selection of the plants which survived on sick soil. After repeated selections, seed was obtained which produced well on sick soils. Barker studied the problems brought up by the work of Bolley, seeking to find if the resistant strains were pure lines sorted out by the pathogen from a mixed population and if there was the necessity of the continued selection advocated by Bolley and seemingly shown by the early work of the Wisconsin investigators.

Barker eliminated the complications in such problems as

arise from environmental conditions with their profound influence on the amount and type of infection. Then he showed that wilt resistance is not an artificial condition built up by exposure of plants to the disease conditions, but instead selection work, using sick soil, gives resistant genotypes. The greater progress, which results from individual plant selections, comes from the closer selection. Resistance of flax to *Fusarium lini* is, according to Barker, a heritable entity comparable with any other genetic character.

A similar story could be told of the development by Essary, Edgerton,¹⁵ and Pritchard of tomatoes resistant to tomato wilt, work of great significance to agriculture.

But it is a speaker's privilege to bring in the work of his own laboratory, and as corroborative evidence I wish to report on our work with the *Fusarium* wilt of celery which we have called "celery yellows."

In 1914 the yellows disease of celery was found at Kalamazoo, Michigan, and gave every indication of being a disease caused by a soil-inhabiting parasite, extremely persistent in soil. In collaboration with Mr. Ray Nelson, we have shown that this disease is due to a species of *Fusarium* and that it operates on these plants by invading the vascular systems and poisoning the plants by some unknown toxic substance. By 1918 the entire district devoted to celery at Kalamazoo showed almost uniform infestation and "golden self-blanching celery," the susceptible variety, was a complete failure in the district.

The situation was first met by securing the substitution of "easy blanching celery," a resistant green celery, for the golden

self-blanching, and in a year the entire district turned to this variety.

In diseased fields we noted, from the first, occasional plants which produced marketable plants, but the vast majority of these was green celery, for commercial seed of celery is deplorably mixed. Repeated selections were made in the fields with the severest cases of infestation of plants of the golden self-blanching type which had produced marketable plants, but these plants rotted in storage. This came about because the resistance shown by the plants selected was of the nature of tolerance, after invasion, to the toxic substances from the invader, but such plants fell ready victims to soft rots during winter storage. Finally, in 1919, Mr Nelson was able to bring one especially fine golden self-blanching plant through the winter, by transplanting to the greenhouse, and seed was obtained. This seed and reselections have been tested on infested soil repeatedly, and all plants showing any tendency to the production of green types have been rejected.

As a result of several years' work we have been able to produce a type of golden self-blanching celery with high resistance to this *Fusarium* disease. The seed has been tested on many soils, and we have had enthusiastic comment from some growers.

Although in the last ten years there has been considerable advance in the development of disease-resistant varieties in other groups, notably the rusts and smuts, the advance along this line and the production of commercial varieties suitable for use under disease conditions have been most conspicuous with disease of the *Fusarium* wilt type.

We are as yet in the dark as to the nature of disease resistance in plants, but it certainly is a far from simple thing, and the cases included under the category vary enormously in the fundamental quality which makes them resistant

The resistance of plants to *Fusarium*, so far as we know it, is rather a tolerance to the fungous invasion, coupled sometimes with a walling off of the invader. It is a relative thing, upset by the extremes of temperature and other environmental factors, especially when the host is in the seedling stage. It would be hazardous, to be sure, to apply too broadly the analysis of the *Fusarium* researches to similar work with other plants and other diseases. But two important characteristics of work in breeding *Fusarium*-resistant plants can be brought out, to the advantage of breeding work for disease resistance in general. These are very simple points, but the wholesale disregard of them in the work with other diseases emboldens me to mention them.

In the first place, in the selection work with *Fusarium*, it has been possible to make selections under conditions of almost 100 per cent infestation, and we are led without delusion to pick the plants which have real potentialities of giving a disease-resistant progeny. In the case of the selections so often made in breeding work for resistance to other plant diseases, care is not taken to provide such severity of infection that mistake is not made in selecting the parent stock. With the selection of merely lucky plants or plants but slightly resistant we fail in our purpose.

And second, in the work with *Fusarium* wilt we have, unconsciously to be sure, dealt not with a single parasitic entity

such as we might select and grow under laboratory conditions, but we have been able to make the selections in the fields where plants have been exposed to a considerable range of pathogenic forms which fall within the *Fusarium* species with which we have been concerned, and thus our selection, tested to manifold types in nature, has greater possibilities of succeeding under diverse conditions and with different strains of the fungus than if the selection had been based on the more restricted testing which has been so often employed in other pathologic work

Confronted by a group of parasites whose importance to agriculture warranted giving them the closest study, there has developed an intense research on the pathogens, with the result that workers have not been able in their general work in plant pathology to keep pace with the developments in the taxonomy of the group. The writer has sought to bring before you the results of work, as yet in progress, which in the tangled condition of the genus have some promise of service to the studies now occupying workers in plant pathology

We have found that use of sera from animals injected with *Fusarium* materials as antigens, yields clean-cut and decisive results once the sensitization is accomplished, and for those that are able to provide the facilities, the methods of the serologist offer opportunities. The aniline dye media and the utilization of the great variety of response of the organisms of this group to the toxic substances, offer a ready tool for use in the particular problems confronting the worker. If I have any contribution to make in this discussion, it does not

lie in the advocacy of the particular methods outlined, although I believe in them, but it is this, that workers in plant pathology confronted with new problems must seek new technic from related branches of science to help them

From the vexing side of the story I have turned to the bright side, and it has been my privilege to tell you in a sketchy way the success plant pathology has had in the development of *Fusarium*-resistant crops. This work has been of great importance to agriculture, and in it we can see the great possibilities for the future.

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RACIAL SPECIALIZATION IN PLANT DISEASE FUNGI*

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INTRODUCTION

Plant diseases are of tremendous importance to every one of us, although all of us may not realize it. Human subsistence depends on plant growth. What we eat and wear comes either directly or indirectly from plants. So also does much of what we use for building our houses and for means of transportation and communication. Plant diseases annually destroy enor-

* Lecture given at the University of Wisconsin, Madison, Wisconsin, March 14, 1927, State University of Iowa, Iowa City, March 16, and The Mayo Foundation, Rochester, Minnesota, March 17.

† In a lecture of this kind it is, of course, impossible to discuss all of the investigations which have built up our present knowledge of racial or physiologic specialization in fungi. I have, therefore, selected those which seemed to illustrate best the ideas I wished to convey. And some have been selected in preference to others equally good because I was more familiar with them. A fairly extensive, but by no means complete, bibliography has been added, in hope that it may be useful.

Thanks are due many of my colleagues at the University of Minnesota and the United States Department of Agriculture for the use of unpublished data and for help in preparing the paper. I wish to thank the following for critical reading of the manuscript and for numerous suggestions: Dr J. J. Christensen, Dr W. F. Hanna, Dr M. N. Levine, Dr O. S. Aamodt, Dr H. A. Rodenhiser, Dr E. B. Lambert, and Dr W. N. Ezekiel. Dr Christensen and Dr Hanna also helped to prepare the Bibliography.

mous quantities of potential or actual foodstuffs, clothing, and building materials. The science of plant pathology, therefore, contributes greatly to human welfare by studying plant diseases and devising methods for preventing or controlling them. In order to do this, it is obviously necessary to know intimately not only the crop plants whose growth we are trying to promote, but also the parasitic plants whose growth we are trying to prevent. And it is of prime importance to know not only what these plant pathogens look like structurally, but also how they behave physiologically. To understand the behavior of plant pathogens one must know, among other things, the characteristics of the physiologically specialized races or forms which many of them comprise.

THE MEANING OF PHYSIOLOGIC SPECIALIZATION

What does physiologic specialization mean? Merely this. Within morphologic species there are entities, not readily distinguishable by structure, which differ from each other physiologically. The entities have been called *Formæ speciales*, *Schwesterarten*, *Gewohnheitsrassen*, biologic species, biologic forms, physiologic races, specialized races, parasitic strains, racial strains, and physiologic forms. In this paper the term "physiologic form" will be used. Whatever the designation, the underlying idea has been that there are within many species of fungi definite entities which cannot be distinguished from each other readily by morphologic characters but are readily separable from each other by their physiologic behavior, including ability to infect certain genera, species, or

varieties of higher plants. For example, powdery mildew affects wheat, oats, barley, rye, and many cultivated and wild grasses. The mildew looks the same to the casual observer, regardless of the plant on which it may be. And when the mildew is examined with the aid of the microscope it still looks essentially the same whether it comes from wheat, oats, barley, rye, or any of the other grains or grasses. The general structural characters are the same, and therefore one must conclude that the mildew from all of the different cereals belongs to one botanical species. And that is exactly where it does belong. It is designated by the name *Erysiphe graminis* D C. But if wheat is inoculated with the mildew from oats, no infection results, and if oats is inoculated with the mildew from wheat, no infection results. In general, plants become mildewed only if inoculated with spores of the mildew produced on plants of the same kind. Within the species *E. graminis*, then, there are parasitic races or strains which look alike, but which have different infective capacities. These races or strains cannot be distinguished from each other by their appearance, but they can be distinguished readily by their ability to infect certain host plants and, in certain fungi, by other physiologic characters. They are, therefore, called physiologic races or physiologic forms.

HISTORICAL SUMMARY

Schroeter, in 1879, was one of the first to observe that there are physiologic forms of plant pathogenic fungi. However, Eriksson,⁴⁷ a Swedish mycologist and plant pathologist, was the first to demonstrate in a clear-cut and definite manner the

very decided physiologic differences between physiologic forms of the same species. Previous to Eriksson's investigations the black stem rust of the cereal grains and grasses was considered to be caused by one species of fungus, *Puccinia graminis* Pers. It was known that *P. graminis* caused black stem rust on wheat, oats, barley, rye, and a large number of cultivated and wild grasses. However, Eriksson showed that the rust from wheat would infect wheat, but not oats, that from oats would infect oats, but not wheat and barley, that from rye would infect rye and barley, but not wheat and oats. He showed, also, that there were parasitic strains of the fungus on different wild grasses. Thus he demonstrated clearly that the morphologic species, *P. graminis*, actually comprises several physiologic forms which differ from each other but little morphologically, but whose infection capabilities are quite distinct. A few years later, Neger in Germany, Marchal in Belgium, and Salmon¹¹⁶ in England showed that the powdery mildews of cereal grains and grasses also comprised different physiologic forms. For several years the phenomenon was supposed to be more or less peculiar to the rusts and powdery mildews. Many investigators made exhaustive researches on the physiologic specialization in these two groups of fungi. But it is now known that physiologic specialization is very common in many widely separated groups of fungi, including the following: The rust fungi; the powdery mildews; the smut fungi; many of the fungi causing root rots and stem rots of small grains, *Colletotrichum lindemuthianum*, the bean anthracnose fungus; *Septoria* spp., which cause leaf spots on many different kinds of plants, and

a very large number of others * In fact, physiologic specialization occurs in so many different species of fungi that one is inclined to wonder whether practically all fungi, at least those parasitic to higher plants, do not in reality comprise many physiologic forms

METHODS OF RECOGNIZING PHYSIOLOGIC FORMS

There are three principal methods by which physiologic forms can be recognized (1) By pathogenicity for certain selected plants, (2) by cultural characters on artificial media, and (3) by physicochemical reactions In addition, there are slight, though statistically significant, morphologic differences between some forms, and these differences sometimes aid in the identification of the forms.

Pathogenicity—Many physiologic forms can be recognized best by their pathogenicity for certain plants Indeed, some of them can be recognized readily only in this way This is true particularly of the rust fungi and the powdery mildews, which apparently are obligate parasites, and have never yet been grown on artificial media Probably the most extensive investigations of physiologic specialization have been made on *Puccinia graminis*, the pathogene which causes black stem

* For a summary of investigations up to 1918 see Reed ¹¹⁰ References to many of the papers published since 1918 will be found in the bibliography at the end of the present paper The following deal largely with the demonstration of specialization 8, 9, 10, 11, 13, 14, 15, 23, 24, 25, 31, 32, 34, 35, 36, 40, 46, 47, 48, 49, 51, 52, 53, 55, 57, 63, 67, 72, 73, 76, 81, 82, 87, 88, 92, 93, 94, 95, 97, 98, 99, 101, 102, 105, 107, 109, 110, 111, 112, 113, 114, 116, 118, 119, 120, 123, 124, 126, 129, 130, 131, 133, 135, 138, 146

rust of grains and grasses, and the method of determining physiologic forms of this pathogene is therefore given in some detail *

Two categories of parasitic strains are recognized in *Puccinia graminis*. Varieties and physiologic forms. Formerly these parasitic strains were known by several names. But, for the sake of clearness, it seems better to use only the terms "variety" and "physiologic form."

The varieties differ from each other somewhat in size and shape of spores^{56, 128}. Some of them can be distinguished by morphology alone. But the principal difference between them is their parasitic capability on host plants. Each variety can attack several species of one or more genera of the grass family, but it cannot attack the members of other genera which may be quite susceptible to other varieties of the rust fungus. A variety in turn may comprise several physiologic forms which differ from each other principally in their ability to attack varieties within one or more species of a genus. Varieties are given Latin names, and physiologic forms are designated by Arabic numerals. Thus, *P. graminis tritici* 18 means physiologic form 18 of the variety *tritici* of the species *P. graminis*. Table 1 gives a general idea of the parasitic specialization within *P. graminis*.

It will be seen from Table 1 that there are six varieties of *P. graminis* in the United States: *Tritici*, *secalis*, *avenæ*, *phlei*-

* This summary is based on results obtained by the writer and his colleagues in cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota.

RACIAL SPECIALIZATION IN PLANT DISEASE FUNGI

TABLE 1

VARIETIES OF THE SPECIES PUCCINIA GRAMINIS IN THE UNITED STATES WITH THE PRINCIPAL HOST PLANTS FOR EACH AND INDICATING PHYSIOLOGIC SPECIALIZATION WITHIN THE VARIETIES

Variety	Host plants †	Physiologic forms
<i>tritici</i>	Wheat, barley, rye, ‡ Agropyron spp § (A repens, very resistant), Bromus spp, Elymus spp, Hordeum spp, Hystrix patula	More than fifty, differing in their effect on twelve varieties of Triticum spp
<i>secalis</i>	Rye, barley, and same grasses as above (Agropyron repens very susceptible)	About a dozen, differing in their effect on five varieties of rye
<i>avenae</i>	Oats, Agrostis exarata, Alopecurus spp, Anthoxanthum spp, Arrhenatherum elatius, Bromus tectorum, Calamagrostis canadensis, Dactylis glomerata, Holcus lanatus, Koeleria cristata, Panicularia pauciflora, Phalaris spp	Five or six, differing in their effect on three varieties of oats
<i>phleipratensis</i>	Timothy, oats, ‡ barley, ‡ Alopecurus spp, Dactylis glomerata, Festuca spp, Holcus lanatus, Koeleria cristata.	None demonstrated.
<i>agrostis</i>	Agrostis spp and grasses listed under phleipratensis, except Festuca spp	None demonstrated
<i>poae</i>	Poa spp	None demonstrated.

* Further details are given in the following references 9, 87, 124, 129, 130, 131, 133

† Achyrodes is fairly susceptible to all varieties.

‡ Infection usually weak.

§ Where several species are indicated, it does not follow that all species of the genus are susceptible, where only one species is indicated, it does not necessarily mean that other species may not be susceptible

pratensis, *agrostis*, and *poæ* All of them, except variety *phleipratensis*, produce æcia on the common barberry Of the common small grains, wheat, in general, is susceptible only to the variety *tritici*, oats to variety *avenæ*, and slightly to variety *phleipratensis*, rye to variety *secalis*, and slightly to variety *tritici*, and barley is completely susceptible to varieties *tritici* and *secalis*, and slightly susceptible to variety *phleipratensis*, but quite resistant to the other two varieties, *agrostis* and *poæ* This explains why one kind of grain may be heavily rusted in the field while other kinds nearby may be relatively free It also explains why barberry bushes, the alternate host of *P graminis*, often may be heavily rusted while grain very near to them may be uninjured

Each of the varieties *tritici*, *avenæ*, and *secalis* consists of several physiologic forms which differ in their ability to attack certain varieties within *Triticum* spp, *Avena* spp, and *Secale cereale*, respectively For instance, more than fifty physiologic forms of the variety *tritici* have been distinguished by their parasitic effect on twelve so-called differential varieties of common and durum wheats, emmers, and einkorn A variety of wheat may be immune from some forms, highly resistant to some, moderately susceptible to others, and completely susceptible to still others (Fig 1)

The method of determining physiologic forms of the *tritici* variety is given in detail The determination of the forms of the *tritici* variety is based on their effect on the following varieties of *Triticum* spp ¹²⁰

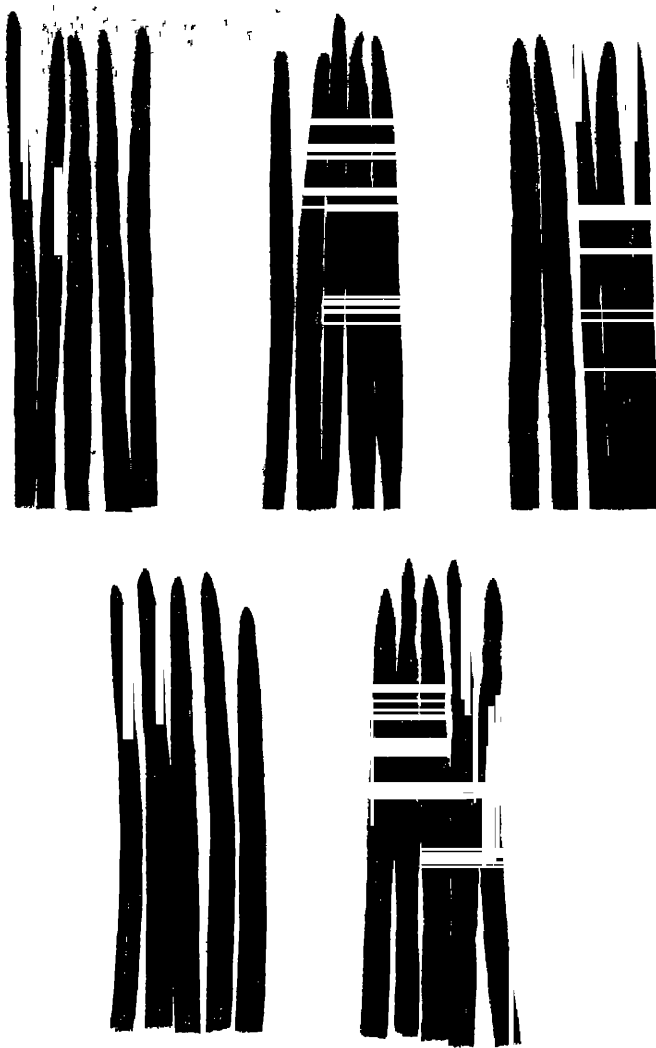


FIG 1—Marquis wheat inoculated with five physiologic forms of the wheat stem rust fungus, *Puccinia graminis tritici* (each set of five leaves was inoculated with a separate form) The range of reaction to rust is shown, from immunity on left above to complete susceptibility on right below

DIFFERENTIAL HOSTS USED IN IDENTIFYING PHYSIOLOGIC FORMS OF
STEM RUST OF WHEAT*Triticum compactum*

Little Club, C I * No 4066

Triticum vulgare

Marquis, C. I. No 3641 (Minn 1239)

Kanred, C. I. No. 5146 (Kans 2401)

Kota, C I No 5878 (N. D 10,003)

Triticum durum

Arnautka, C I. No. 4072 (S D 150)

Mindum, C I No 5296 (Minn 470)

Spelmar, C. I. No. 6236 (Minn 337) .

Kubanka, C I No. 2094

Acme, C I No 5284 (S. D 284)

Triticum monoccum

Einkorn, C I No 2433

Triticum dicoccum

Vernal, C I. No. 3676 (Minn 1165)

Khapli, C I No 4013

When these differential hosts are inoculated, there are different resultant types of infection. Levine and I have recognized six so-called infection types, which are indicated in Table 2 and Figure 2. If one variety of wheat is inoculated with five different physiologic forms, five different infection types may result; and, if five varieties are inoculated with one form, the same five types of infection may appear. These infection types are described in Table 2.

* C I = Cereal Investigations accession number, United States Department of Agriculture

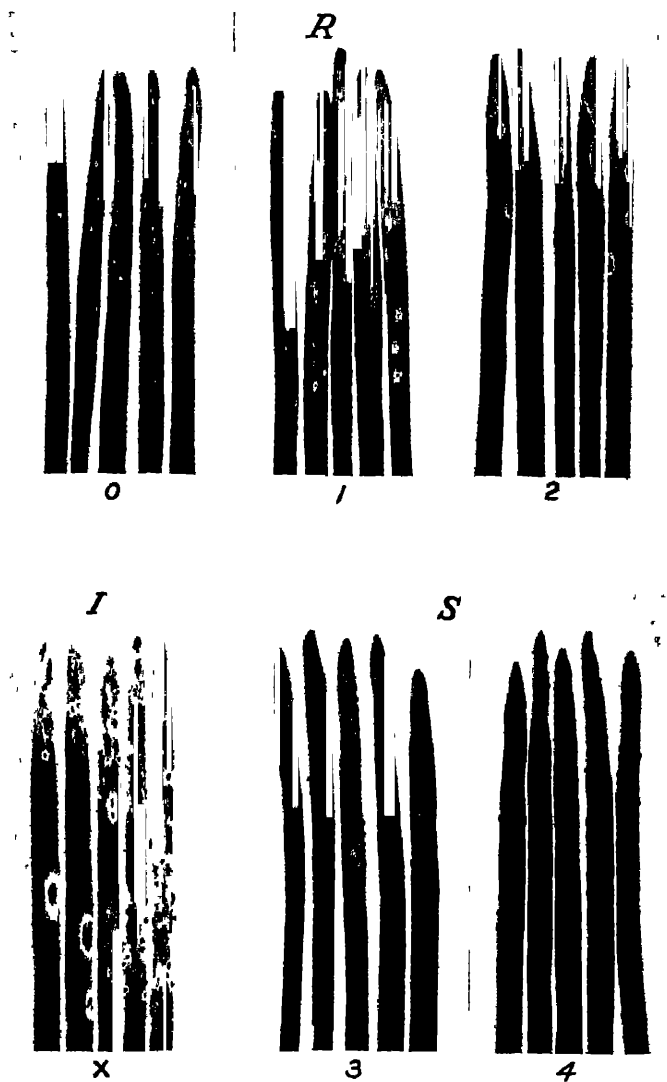


FIG 2—Six types of infection caused by different physiologic forms of the wheat stem rust fungus on "differential" varieties of wheat, emmer, durum, and einkorn. In the analytical key, Table 3, the upper three are grouped under "resistant" and the lower three under "susceptible."

TABLE 2

EXPLANATION OF SYMBOLS USED TO INDICATE TYPES AND DEGREES OF INFECTION PRODUCED BY PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI ON DIFFERENTIAL VARIETIES OF TRITICUM SPF

Types of Infection

(0) Immune

No uredinia developed, hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues

(1) Very resistant

Uredinia minute and isolated, surrounded by sharp, continuous, hypersensitive, necrotic areas

(2) Moderately resistant

Uredinia isolated and small to medium in size, hypersensitive areas present in the form of necrotic halos or circles, pustules usually in green, but slightly chlorotic, islands

(3) Moderately susceptible

Uredinia medium in size, coalescence infrequent, development of rust somewhat subnormal, true hypersensitiveness absent, chlorotic areas, however, may be present, especially under unfavorable cultural conditions

(4) Very susceptible

Uredinia large, and generally confluent, true hypersensitiveness, entirely absent, but chlorosis may be present when cultural conditions are unfavorable

(X) Heterogeneous

Uredinia very variable, apparently including all types and degrees of infection, often on the same blade, no mechanical separation possible, on reinoculation small uredinia may produce large ones, and vice versa

Degrees of Infection

(=) Trace

Uredinia very few in number and covering a limited surface, development of rust generally poor and decidedly subnormal

(-) Slight

Rust development below normal, but somewhat better than "trace"

(±) Moderate

Variation in rust development from "slight" to "considerable", when infection is uniform but only medium in quantity the symbol is omitted

(+) Considerable

Infection better than normal, uredinia fairly numerous and scattered

(++) Abundant

Luxuriant development of rust, uredinia very many, covering large area of affected host

Miscellaneous Symbols

- (.) Hypersensitive flecks
(.) Necrotic lesions

Therefore, in identifying physiologic forms of *P. graminis tritici*, it is necessary to inoculate the differential hosts with the proper collection of rust and refer to the analytical key which is shown in Table 3

TABLE 3

ANALYTICAL KEY TO PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS WITHIN
THE GENUS TRITICUM

Infection homogeneous on all differential hosts

Marquis resistant (R)

Kanred resistant

Kota resistant

Arnautka resistant

Kubanka resistant

Form 2

Kubanka susceptible

Einkorn resistant

27

Einkorn susceptible

23

Arnautka susceptible

Mindum resistant

6

Mindum susceptible

Kubanka resistant

4

Kubanka susceptible

Einkorn resistant

16

Einkorn susceptible

14

Kota susceptible

Mindum resistant

28

Mindum susceptible

19

Kanred susceptible

Arnautka resistant

Kubanka resistant

7

Kubanka susceptible

33

Arnautka susceptible

10

Marquis susceptible (S)

Kanred resistant

Kota resistant

24

Kota susceptible

Infection homogeneous on all differential hosts	
Arnautka resistant	1
Arnautka susceptible	
Mindum resistant	26
Mindum susceptible	
Kubanka resistant	
Vernal resistant	5
Vernal susceptible	8
Kubanka susceptible	
Einkorn resistant	21
Einkorn susceptible	
Vernal resistant	17
Vernal susceptible	9
Kanred susceptible	
Kota resistant	35
Kota susceptible	
Arnautka resistant	
Mindum resistant	
Kubanka resistant	3
Kubanka susceptible	
Acme resistant	20
Acme susceptible	18
Mindum susceptible	
Spelmar resistant	25
Spelmar susceptible	22
Arnautka susceptible	
Mindum resistant	12
Mindum susceptible	
Kubanka resistant	13
Kubanka susceptible	
Einkorn resistant	34
Einkorn susceptible	
Vernal resistant	11
Vernal susceptible	15
Infection heterogeneous on some differential hosts	
Marquis susceptible	
Kanred resistant	
Kota susceptible	
Mindum indeterminate (X)	
Vernal resistant	29
Vernal susceptible	30
Mindum susceptible	
Kubanka indeterminate	37

Infection heterogeneous on some differential hosts

Kanred susceptible

Kota resistant

Mindum indeterminate 31

Kota susceptible

Mindum resistant

Kubanka indeterminate 36

Mindum indeterminate 32

By means of this key it is possible, then, to determine tentatively the identity of the form. The behavior of the rust collection in question is then compared with that of the form which it is supposed to represent, and, if it agrees in all particulars, the identification is complete. If it does not agree, either there may be a mixture of forms in the collection, or the collection may represent a new form. Further inoculations are then required.

The constancy in the behavior of the physiologic forms of *P. graminis tritici* is quite remarkable. There are, of course, fluctuations in the degree of infection, depending on the environmental conditions under which the rust develops.^{106, 128} But these fluctuations are no greater than, possibly not so great as, those in the morphologic characters of fungi grown under different environmental conditions. The determination of physiologic forms is quite as definite and precise as is the determination of morphologic species. The high degree of specificity in their intimate relations with species and varieties of higher plants also is noteworthy.^{4, 5, 6, 125} In fact, the more one investigates physiologic forms in the rust fungi, the more he becomes impressed with the fact that they are quite as real and constant entities as are the species and varieties of host plants on which they grow.



FIG 3—Seedlings of Marquis wheat grown in soil inoculated at time of planting with *Helminthosporium sativum*, showing the comparative virulence of different forms of the pathogene A, Uninoculated, B, form 26, C, form 21, D, form 3, E, form 19, F, form 22, G, form 8, H, form 5

There are numerous physiologic forms in a great many other rust fungi. Orange leaf rust of wheat⁹²; yellow stripe rust of cereals,⁹⁸ crown rust of oats,^{67, 95, 105} sunflower rust,⁸ and a long list of others. To discuss them all in detail would require too much space. In general, however, they can be identified quite as readily as can those of *P. graminis*.

It has not been possible to determine infection types with so great a degree of precision in the case of certain other fungi. However, the existence of these forms can be demonstrated readily by inoculating different varieties of plants. For example, there are many different physiologic forms of *Helminthosporium sativum*, a destructive pathogene on wheat, barley, rye, and many grasses. Christensen³⁶ has studied thirty-seven in detail. Some of them are very virulent on certain hosts, some moderately so, and some of them are relatively innocuous, although they are all practically identical morphologically (Fig. 3). The same is true of *Fusarium lini*, the fungus which causes flax wilt. Broadfoot has studied this fungus carefully and has shown that there are at least eight physiologic forms which can be recognized by their parasitic effect on varieties of flax. One of them is unique in that it produces chlorosis of the infected plants (Fig. 4). There are also many physiologic forms of the fungi causing the smuts of oats, that causing the stinking smut or bunt of wheat, and of the fungi causing corn smut and smuts of sorghum.^{30, 111, 112, 113, 138}

Reed¹¹¹ showed that certain varieties of oats which were practically immune from oat smut in the United States were completely susceptible to some forms of the same smut which

he obtained from Europe Rodenhiser and Stakman have shown that there are distinct forms both of *Tilletia levis* and *T. tritici*. These forms can be recognized readily by their effect on certain varieties of wheat (Fig. 5). Stephens showed that certain varieties of wheat grown in Oregon were very resistant to a form of bunt (*T. tritici*) in Oregon, but were quite susceptible to another form which was collected in the same state Stakman and Christensen,¹²⁶ and Christensen



FIG. 4.—The plants on the left show the chlorotic effect of one physiologic form of the flax wilt fungus. The plants on the right were not inoculated.

and Stakman showed that there are many forms of *Ustilago zeæ*, the organism causing corn smut. Certain self-pollinated lines of corn were very resistant to some of these forms, but completely susceptible to others (Fig. 6).

There are so many physiologic forms of many fungi which cause plant disease, and some of them have so high a degree of specificity for certain varieties of crop plants, that one is scarcely justified in designating a variety as resistant without specifying the physiologic forms to which it is resistant.

variety may be immune from some forms, moderately resistant to certain others, and completely susceptible to still others (Fig 1) And the identification of these forms by means

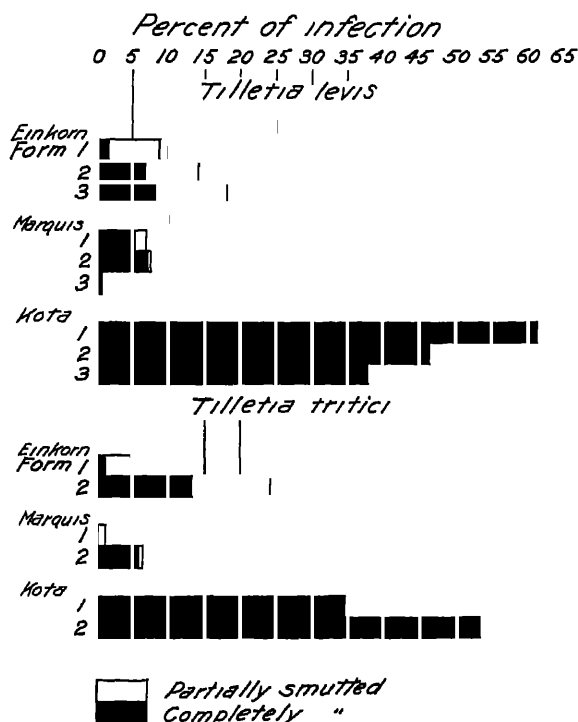


FIG 5—The relative amounts of bunt produced in Marquis and Kota wheats and einkorn by different physiologic forms of *Tilletia levis* and *tritici*. Form 1 of *T levis* was collected in Hungary, form 2 in Minnesota and form 3 in Egypt. Form 1 of *T tritici* was collected in New Zealand and form 2 in Norway.

their effect on differential hosts often is quite as precise as the determination of chemicals by standard reagents.

Cultural characters—Physiologic forms of certain fungi can



FIG 6—The effect of two physiologic forms of the corn smut fungus on a supposedly resistant line of corn, Rustler (Culture 4400—1925), selfed six years. This line of corn is almost immune to form 7 (below), but extremely susceptible to form 8 (above)

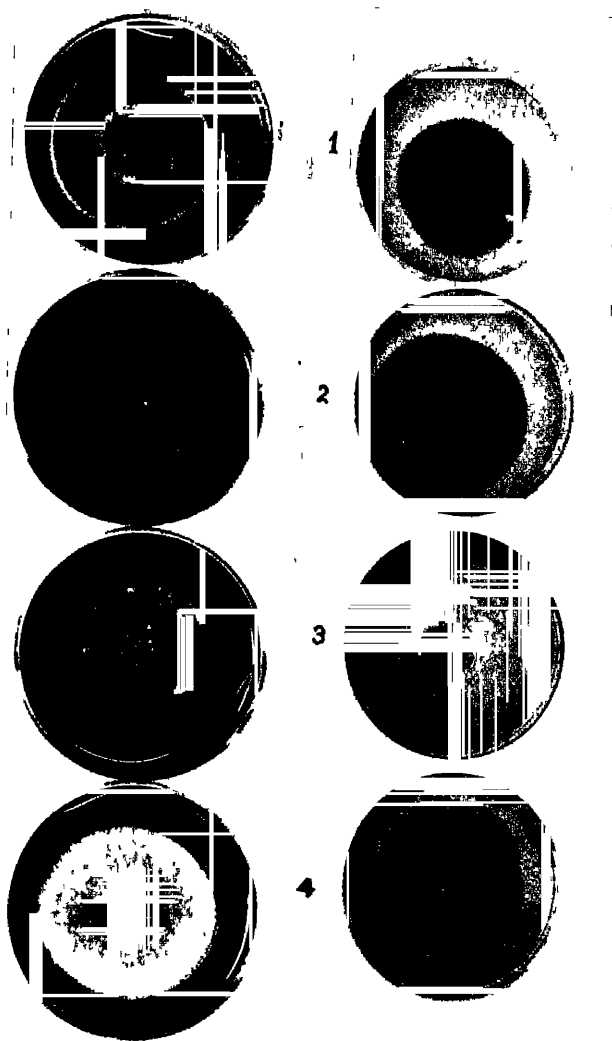


FIG 7—Four physiologic forms, 1, 2, 3, and 4 of *Helminthosporium sativum*. Left, on green bean agar, right, on potato dextrose agar. The same form grown on different media may look like two different fungi, but the appearance of a given form is remarkably constant for given conditions.

be recognized readily by their growth characters and biochemical reactions on certain media. Christensen³³ has shown that forms of *Helminthosporium sativum* can be recognized readily in this way. When grown on the same medium, some of the forms look so unlike that one would scarcely think they belonged to the same species. The cultural characters of a given form of this particular fungus are quite constant when grown under uniform conditions,



FIG 8—Three physiologic forms of *Sorosporium reikhanum* (kernel smut of sorghum) growing on the same medium. On left, from Egypt, center, from Iowa, on right, from Minnesota

but cultures of a single form grown on different media may look very unlike (Fig. 7). If one grows the same physiologic form on three different media, the appearance may be that of three distinctly different species of fungi. But yet the morphology of the spores may be approximately the same on all of the different media. What is true of *Helminthosporium sativum* also is true of many other fungi. *Ustilago zeæ*, *U. tritici*, *U. nuda*, *U. avenæ*, *U. levis*, *Sorosporium reikhanum*, *Sclerotinia cinerea*, *S. americana*, and a large num-

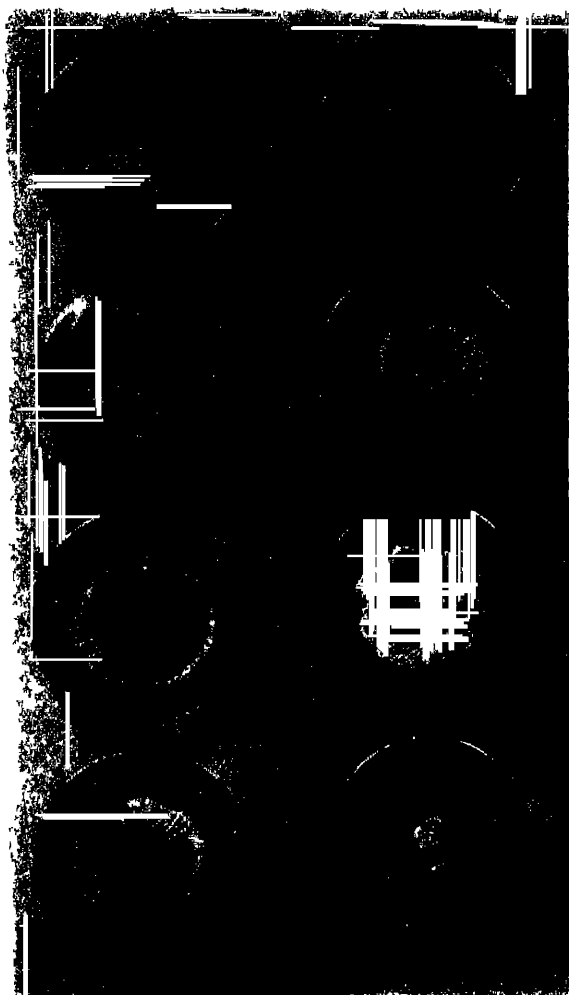


FIG. 9.—Eight physiologic forms of *Ustilago zeæ* (corn smut) growing on potato-dextrose agar.

ber of other fungi comprise physiologic forms which can be recognized readily by their cultural characters on artificial media (Figs 8, 9, and 10)

The first indication of physiologic specialization often can be obtained by differences in the cultural characters of different forms. This fact is of considerable importance, since it enables pathologists to make cultures of collections of the

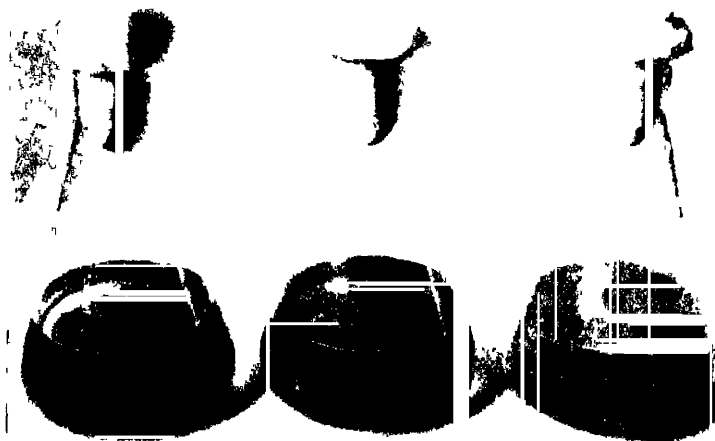


FIG 10—The effect of three physiologic forms of *Ustilago zeae* (corn smut) in dextrose-carrot extract. These three forms can be distinguished from each other by the degree to which they discolor the medium.

same pathogenic fungus from different localities and different host plants and distinguish between them on the basis of their cultural characters. Inoculations can then be made with the different cultures, and it is relatively easy to ascertain whether the physiologic forms differ also in pathogenicity.

It would obviously be a tremendous advantage in phytopathologic work if one could always be sure from the appear-

ance of a pathogene in culture what its pathogenic capabilities might be. In some cases this is possible, but as yet the cultural test cannot generally be substituted for the pathogenicity test. After all, the principal object of determining physiologic forms is to give the investigator assistance in pathologic investigations by ascertaining the pathogenic abilities of the fungi which cause plant disease. So far the culture test has been valuable in giving the first clue to possible differences.

Physicochemical reactions—Physiologic forms, as the term implies, also may differ from each other in their physicochemical reactions. In fermentative ability, temperature requirements, and reaction to hydrogen-ion concentration.

Dox and Thom showed that strains of fungi might differ considerably in their enzyme activity. Letcher and Willaman showed that forms of *Fusarium lini*, the organism that causes flax wilt, differ considerably in their ability to produce alcohol from certain sugars. As a matter of fact, there seems to be fairly close correlation between ability of the different forms to produce alcohol and their virulence for host plants, as shown by inoculations made by Broadfoot.

It has been known for some time that closely related forms of fungi may have different temperature requirements. Edgerton⁴⁵ found that certain strains of *Glomerella* differed considerably in their temperature relations. Hursh⁶⁰ found that the optimal temperature for germination of the urediniospores of different physiologic forms of *Puccinia graminis tritici* differed considerably. Weimer and Harter, and Harter and Weimer showed that there are many strains of *Rhizopus nigricans*, and they found at least one that could be dis-

tinguished readily from all of the others by means of its reaction to temperature. At Minnesota we have found that different physiologic forms of smut fungi may differ greatly in relation to temperature (Fig 11). Johnson was able to distinguish two physiologic forms of the barley stripe pathogene (*Helminthosporium gramineum*) by their reactions to temperature



FIG 11—*Ustilago tritici* (loose smut of wheat) grown at 30° C on potato-dextrose agar: Left, form 1, from Minnesota, right, form 2, from Egypt

It has been shown also that physiologic forms which differ from each other in their parasitic ability may have different degrees of tolerance for hydrogen-ion concentration Hursh⁶⁰ demonstrated this quite clearly for physiologic forms of *P graminis tritici*.

It is obvious, therefore, that within morphologic species of fungi pathogenic to plants there may be numerous physiologic

forms which are practically indistinguishable in appearance, but which may differ greatly and consistently in pathogenicity, in cultural characters, and in certain physicochemical reactions

How did these forms originate? How constant are they? And what is their significance? These questions are not only of scientific interest, but also of great practical importance.

ORIGIN AND CONSTANCY OF PHYSIOLOGIC FORMS

The question of the origin of physiologic forms is probably not greatly different from that regarding the origin of morphologic species. However, for those concerned with the growing and improvement of plants, the question is not only of academic interest, but also of the greatest practical importance

There have been three general ideas regarding the origin of physiologic forms: (1) Adaptation or so-called education; (2) hybridization, and (3) mutation *

For many years it was supposed that physiologic forms could be changed easily by altering environmental conditions. It was supposed that the pathogenicity could be changed at will by two methods: (1) The gradual adaptation of physiologic forms to resistant hosts and (2) by means of bridging species. It was supposed that if a resistant plant were inoculated with a certain physiologic form, the resulting infection would be rather weak, but if the fungus were transferred a second time to the same host, it would acquire additional virulence, and,

* For a comprehensive discussion see Brierley ^{20, 22}

if it were transferred repeatedly to the same host, it would gradually acquire the ability to attack it normally. Ward, for instance, made experiments with the brown rust of bromes, *P. dispersa*, which he thought demonstrated that the rust might gradually adapt itself in this way to resistant hosts. Furthermore, he thought he had evidence that certain species or varieties might act as a bridge between susceptible and immune species. He pictured the process approximately as follows with the brome rust. The rust was able to attack species A and species B, but not species C. Ward thought that by transferring the rust from A to B, intermediate taxonomically between A and C, it would acquire the ability to attack the normally immune species C. That is, species B acted as a bridge, enabling the rust from A to attack C, which it could not do without first having been grown on B. Salmon¹¹⁷ drew similar conclusions with respect to the powdery mildews of cereals. Freeman and Johnson suggested that bridging also was common in *P. graminis*. They stated that the stem rust from wheat could not be transferred directly to oats, but that it could be transferred from wheat to barley and then from barley to oats. In this case, therefore, barley was considered to serve as a bridge over which the rust could pass from wheat to oats. Arthur suggested that the common barberry, on which the cluster-cup stage of black stem rust develops, constituted a bridge which enabled the different physiologic forms of this fungus to attack hosts which normally were resistant or immune. Evans thought that hybrids between resistant and susceptible varieties of wheat might be more susceptible than the susceptible parent and

would act as a bridge enabling the rust to attack even the resistant parent

If the theories of gradual adaptation and bridging were true, physiologic forms would be unstable entities, mere ecads changing rapidly to adapt themselves to new host plants or new climatic conditions. The implication would be that the differences between them were due only to environmental factors, not to genetic factors. The existence of forms would then furnish evidence of remarkable variability or adaptability of pathogenic fungi, but not of a definite parasitic specialization within species.

Can parasitic fungi acquire new pathogenic abilities easily and quickly, merely by growing on certain plants? There is much evidence that they do not. Stakman, Parker and Piemeisel, Stakman and Piemeisel, and Stakman, Piemeisel, and Levine never could obtain any evidence that physiologic forms of *P. graminis* acquired additional virulence for resistant hosts as a result of being propagated on them even for long periods of time. Neither could they obtain any evidence whatever that so-called bridging actually occurred. On the other hand, it was shown that each of the so-called major physiologic forms (now called varieties) of *P. graminis*, namely, that attacking wheat, that attacking oats, and that attacking rye, comprised physiologic forms which could be distinguished by their ability to attack certain varieties of wheat, oats, and rye, respectively.^{87, 120, 131} That is, there are physiologic forms within physiologic forms. The earlier investigators did not know this and it may possibly explain, at least in part, some of their results. It may be possible that

bridging and gradual adaptation to new host plants actually occur, but we never have been able to obtain conclusive evidence for it at the University of Minnesota, although we have investigated the problem for many years.

It began to look as though the ideas of gradual adaptation and bridging had been discarded, but recently Hammarlund concluded that it was possible to increase the host range of *Erysiphe graminis*, the powdery mildew of grains and grasses, by injuring resistant plants. He stated that the mildew from wheat would not infect barley. But when he injured the barley plants, before inoculation, the mildew developed on them, although it did not acquire the ability, while growing on the injured plants, to infect uninjured plants. Levine and I (unpublished results) have made similar experiments. The mildew from wheat infected other wheat plants readily, but it would not infect barley. We therefore injured barley plants and inoculated them, but the mildew did not develop on the injured plants. We injured more than 2,000 plants at many different times, and in many different ways, but none ever became infected with the mildew from wheat.

There must be some reason for the different results obtained in Europe and in the United States. Possibly the European mildew is less stable than that in the United States or possibly the European varieties of wheat and barley are so different from ours as to account for the discrepancies in results. We have tried to make experiments with the European mildew, but unfortunately the spores in most of the collections we have received from Europe were no longer viable and failed therefore to cause infection.

While it would perhaps be unsafe to deny absolutely that physiologic forms gradually adapt themselves to new hosts and that they can attack them as a result of a sojourn on "bridging" hosts, the writer is convinced that the phenomenon does not occur commonly. Physiologic forms do not seem to be unstable in the sense imputed by the terms "adaptation" and "bridging." On the other hand, they seem to be as stable as are many species of higher plants. Of course, they are not irrevocably immutable. They may vary considerably as a result of environmental changes, just as higher plants do, but it remains to be proved that a heritable change can be induced by so-called adaptation or bridging. The differences between physiologic forms are due to differences in genotypic constitution, and when new forms arise they evidently arise as a result of hybridization or mutation, just as higher plants do. This fact was overlooked until comparatively recently, and it seems likely that the observed facts which led earlier investigators to conclude that forms changed their parasitic capabilities readily are to be explained by the fact that there are more forms than they suspected and by the fact that new forms are constantly arising by hybridization and mutation.

Pathogenic fungi may hybridize as higher plants do. It has been known for many years that there are sexual strains of many fungi. These strains may look alike, but behave entirely differently. Blakeslee^{17, 18} showed that zygospores of the common bread mold would not develop unless two strains of opposite sex fused. Dodge demonstrated that the same thing was true for the production of perithecia in *Ascobolus*

magnificus Edgerton⁴⁴ obtained evidence that there were sexual strains of *Glomerella* also. The investigations of Burgeff, Saito and Hirotsuke, Bensaude, Kniep,^{77, 78, 79} Zillig, Buller, Mounce, Hanna, Newton,¹⁰⁰ Bäch,^{12, 13, 14} Seyfert, Vandendries, Derx, Shear and Dodge, and others have given facts pertinent to the problem and proved that there are sexual strains in many fungi. As a matter of fact, it has even been shown that there may be more than two so-called sexes in some of the mushrooms.^{59, 78, 130} This means, of course, that sexual fusions are common, and that different strains are prerequisite to the fusion. Obviously hybridization would be expected between different physiologic forms or even between different species of fungi. Kniep⁷⁹ has shown that such fusions actually do occur between different species of smut fungi, and even that several different species may all fuse together. Couch observed crossing between different species of *Dictyuchus*. Recently, Stakman and Christensen¹²⁷ have shown that inoculation with two strains of opposite sex is necessary, at least in some cases, in order that corn may become infected with the corn smut fungus. These sexual strains were different in other physiologic characters besides that of sex. They differed greatly in appearance on culture media, in biochemical action, and some of them differed greatly in temperature requirements and were therefore physiologic forms. They hybridized on or in the corn plant, thus paving the way for recombination and segregation. Consequently, new physiologic forms may be produced as a result of the hybridization. It is clear from investigations which we have made at the University of Minne-

sota during the last several years that many different species of smut fungi are highly heterozygous and that segregation is constantly occurring. Evidently, therefore, physiologic forms now in existence may have arisen by hybridization, and, what is more important, they still are arising in this way.

Apparently new physiologic forms of parasitic fungi arise by mutation as well as by hybridization. Blakeslee,¹⁹ Burger, Brierley,²¹ Stevens, LaRue, Christensen,³⁴ Leonian, Brown, Christensen and Stakman, and many others have demonstrated mutation.* It is possible that some of the so-called mutations which have been described actually were the result of segregation rather than mutation, but the conditions of some of the experiments were such as practically to preclude this possibility. For example, several of the investigators mentioned have observed mutation in cultures derived from single, apparently asexually produced spores. Mutants have been observed repeatedly in artificial culture, where they appear as pie-shaped sectors in a colony (Fig 12). They may differ in several respects from the parent colony. In color, rate of growth, spore production, and pathogenicity. Christensen³⁶ made an exhaustive study of mutations in *Helminthosporium sativum*. Every possible precaution was taken to insure the genetic purity of the culture. Many of them were the result of a series of successive single spore isolations which produced only asexual spores. Mutants appeared frequently in some physiologic forms and rarely or not at all in others. The frequency of mutation could be controlled somewhat by

* A general discussion on the opposite view of mutation is in Nos. 22 and 26 in Bibliography.

controlling the environmental conditions. Some of the mutants differed from their parents not only in appearance, but also in pathogenicity. Some were less virulent than the pa-

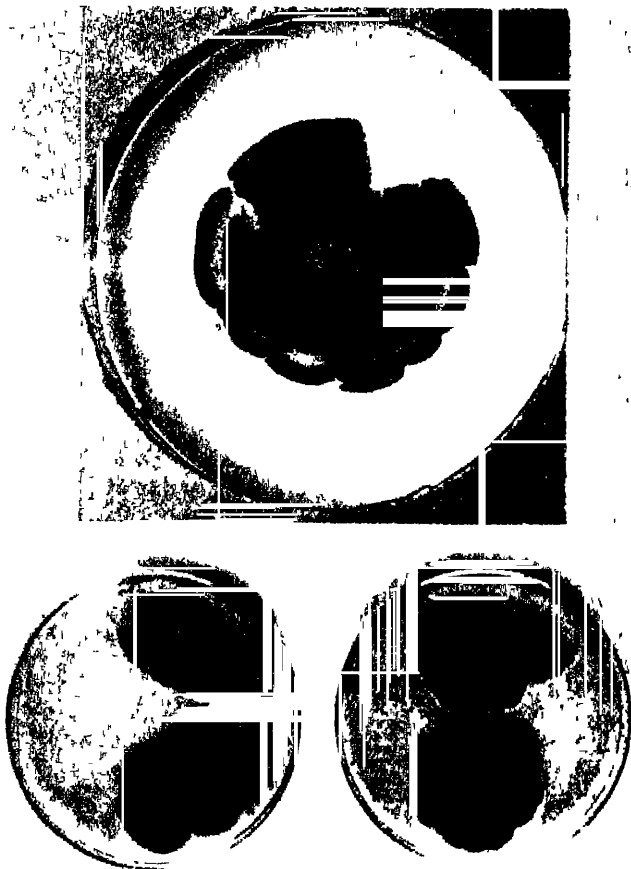


FIG 12—Above, culture of *Helminthosporium sativum*, form 1, on oatmeal-rice-cornmeal agar, showing mutants. Below, a parent and its mutants (light-colored colonies). The mutant had been grown on various media for two and a half years when the photograph was taken.

rent, some about equally virulent, and some were much more virulent (Fig 13)

Until recently mutations never had been observed in the rust fungi and the powdery mildews. As these two groups of fungi cannot be grown in artificial culture, it naturally would be somewhat difficult to detect mutants. However, Newton and Johnson¹⁰⁴ have recently described color mutations in

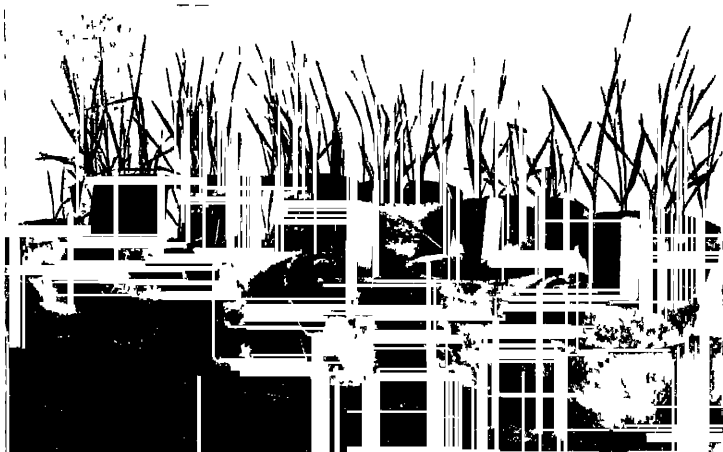


FIG 13—Seedlings of Marquis wheat grown in soil inoculated with *Helminthosporium sativum*, form 22 (back row) and its mutant No 40 (front row). The mutant is much more virulent than its parent.

P. graminis, the organism causing black stem rust on small grains.

Some pathogenic fungi appear to be very heterozygous and are changing rapidly, both as a result of hybridization and mutation. The organism causing corn smut, for instance, seems to be very heterozygous and comprises a large number of different types, many of which may be mutating and hy-

bridizing with each other. Consequently, the organism is very unstable. On the other hand, in spite of the fact that mutations have recently been demonstrated, the fungus causing black stem rust of grains and grasses seems to be relatively stable, although it is by no means absolutely fixed. As an indication of its stability may be mentioned the fact that Levine and I have collected *P. graminis tritici*, form 21, from the United States, Canada, Great Britain, Norway, France, Egypt, and Japan. The parasitic capabilities were remarkably uniform regardless of the country in which it was collected. This probably means that it had been in existence for many years, as it probably would require a very long time for the dissemination from one original source to these widely separated areas.

Generalizations can therefore not be made too freely. The degree of stability differs in different groups. We must know our fungi.

IMPORTANCE OF PHYSIOLOGIC SPECIALIZATION

Physiologic specialization and epidemiology—Many apparently inexplicable facts in the development of plant disease epidemics often can be explained by a knowledge of the geographic distribution and seasonal occurrence of physiologic forms.

It has been known for many years that varieties of wheat may be resistant to black stem rust in one region and susceptible in another. Formerly it was supposed that the resistance actually changed as a result of environmental conditions. However, it is now known that the true explanation

usually is the fact that there are different physiologic forms in different geographic regions, and sometimes even in different localities in the same general region. For example, Marquis wheat is considered very susceptible to black stem rust in the hard red spring wheat region of the Upper Mississippi Valley, but it often is almost free from rust in some of the Gulf states and in certain regions west of the Rocky Mountains. This apparent difference in resistance in different localities is due merely to the fact that most of the physiologic forms of black stem rust which occur in the Upper Mississippi Valley can attack Marquis wheat normally, whereas those in the far South and far West are unable to do so. For many years the durum wheats were much more resistant to stem rust in the Dakotas and Minnesota than the bread wheats. In many years epidemics of stem rust practically ruined bread wheats while the durums were almost uninjured, but in 1923 there was an epidemic on durum, and there was another one in 1927. The reason is simply this: Most of the physiologic forms of the black stem rust which are found in the Upper Mississippi Valley in the average year cannot normally infect most of the varieties of durum. But in 1923 *P. graminis tritici* forms 11 and 17, and in 1927 form 21, which do infect the durums heavily, were very prevalent.

Surveys of physiologic forms actually are being made in rust epidemiology studies by the United States Department of Agriculture, in the United States, and by the Dominion Rust Research Laboratory of Canada, in an attempt to ascertain the source of rust in the spring

One of the very important questions in determining the fac-

tors affecting the development of black stem rust is this. How much rust comes from barberries in the spring wheat area, and how much of it is blown in from the far South, where the rust can persist independently of the barberry? It has been found that in some years the physiologic forms of the wheat stem rust in the South are quite different from those in the North. This is strong circumstantial evidence, therefore, that in those particular years the rust did not migrate from the South to the North, although in other years it may do so.

It seems quite likely that such surveys of physiologic forms can be used in the future for predicting, to a certain extent, the possible development of epidemics of various plant diseases.

Physiologic specialization and plant quarantines—Information regarding physiologic specialization of plant pathogens is fundamental to sound procedure in establishing plant quarantine regulations. It is well known that the physiologic specialization of many plant pathogenic fungi differs in different countries. It is of the utmost importance to realize that one cannot tell by looking at a pathogene what its infection capabilities are. For example, smuts of oats are common in the United States and Europe, as well as in other countries. Then what sense would there be in prohibiting the importation of smut infested seed of oats? More sense than one might realize! The smuts look the same whether produced in Europe or the United States. But the behavior is decidedly different. Reed¹¹¹ inoculated the same varieties of oats with two collections of covered smut of oats (*Ustilago*

levis), one from Wales and one from the United States (Missouri). Here is an example of what happened. Only 2.5 per cent of the plants of *Avena brevis* became infected when inoculated with the smut from Missouri, but 100 per cent of those inoculated with that from Wales became infected. And, just to show that neither country can accuse the other of harboring all the virulent forms, it is recorded that 100 per cent of the plants of the variety Canadian became smutted with the American form of smut, while the Welsh smut caused no infection whatever. Rodenhiser and Stakman showed that there are forms of the fungi which cause bunt of wheat, and the bunt problem in any one country might easily become more acute were new forms introduced into new regions. The same holds true for other smut fungi also. Surely, with such facts before us, the importance of plant quarantines must be evident.

Another example: For a number of years White Tartar oats has been very resistant to black stem rust in the Mississippi Valley of the United States. Valuable rust resistant hybrids, with White Tartar as one of the parents, have been made by plant breeders at the University of Minnesota.⁵⁶ However, White Tartar, as well as these hybrids, is completely susceptible to certain physiologic forms which are known to occur in northern Europe and in South Africa. Should these forms of rust be introduced into the United States and become established here, the value of the breeding work which already has been done for the development of rust-resistant varieties of oats would be largely undone. Still another example may be given. During ten years Levine

and I inoculated Khapli emmer with hundreds of collections of rust from all parts of the United States and many other countries, but never found a collection of rust which would attack it normally. However, we received a collection from Egypt about two years ago and at least two forms isolated from this collection attack Khapli perfectly normally.

It is perfectly clear that many pathogenes which easily could be transported from one country to another in or on seed or other propagative parts of plants comprise many physiologic forms. The mere fact that a certain pathogene has been studied thoroughly in one country is no guarantee that its pathogenicity is sufficiently known. The fact is simply this: The physiologic forms in one country may be relatively harmless to the varieties of crop plants in that country, but they may be very dangerous for the varieties grown in another country. A plant pathogene therefore may be unimportant in one country, but very destructive in another.

Physiologic forms as biologic reagents—Physiologic forms of many pathogenic fungi have an uncanny ability to recognize certain varieties of crop plants. Their ability in this respect is far superior to that of many taxonomic botanists. Therefore, these forms may be useful as biologic reagents in identifying varieties. This is particularly true when the varieties themselves differ principally in physiologic rather than in morphologic characters. Some varieties of plants differ from each other primarily in resistance to disease. They can be identified accurately by inoculation with the proper physiologic form of the pathogene in question. Practical use has been made of this fact by agronomists in Kansas. A number

of years ago, Kanred wheat, which is immune from some forms of black stem rust, was produced and distributed by the Kansas station. Because of its rust resistance and other desirable characters, Kanred is in considerable demand. But how can it be recognized? It is practically impossible to distinguish between Kanred and certain strains of Turkey wheat by their appearance. But here stem rust is a better taxonomist than man. Kanred can be determined with unerring accuracy by simply sowing some seeds of the variety in question in the greenhouse, and inoculating the seedling plants with the proper physiologic form of stem rust. Johnston and Bowers have called attention to the value of this method in the certification of Kanred seed in Kansas. It is very likely that physiologic forms will be used more extensively as biologic reagents in the future. Vavilov,¹⁴¹ Dufrenoy, Aamodt and Levine, and various others have called attention to the preciseness with which varieties can be recognized by their reaction to certain pathogenes. However, as far as I know, the most practical application of the fact has been made in Kansas, as mentioned above.

Physiologic specialization and the development of disease-resistant varieties *—It is absolutely essential to take into consideration the number, geographic distribution, pathogenic peculiarities, and degree of constancy of physiologic forms of a pathogene in attempting to develop varieties of

* The discussion of corn smut is based largely on the coöperative work between the Section of Plant Genetics and the Section of Plant Pathology of the Minnesota Agricultural Experiment Station. The breeding of rust-resistant wheats is a cooperative project between the sections mentioned and the Office of Cereal Crops and Diseases of the United States Department of Agriculture.

crop plants resistant to it Unless this is done, there are bound to be many disappointments in the development of resistant varieties They are likely to be resistant in certain localities and not in others. Even in the same locality they may be resistant in certain years and appear suddenly to become susceptible because of the introduction of a particularly virulent strain of the pathogene. This has been strikingly shown in the case of Kanred wheat, which was immune to stem rust in the particular locality where it was developed. It naturally would be immune there, because the physiologic forms of black stem rust in that locality could not attack it However, there are many physiologic forms in other parts of the country which can attack Kanred perfectly normally, consequently it rusts heavily where and when the forms to which it is susceptible are prevalent We have had a somewhat similar experience at the University of Minnesota during the last few years For several years plant breeders and plant pathologists attempted to produce smut-resistant lines of corn at University Farm, St Paul, Minnesota A number of highly resistant lines were developed. They were very resistant to the physiologic forms of the corn smut fungus which were prevalent at University Farm However, when inoculated with forms collected in other regions of the United States, some of these lines were completely susceptible (Figs 14 and 15)

It is essential to know the reaction of prospective varieties to all of the physiologic forms to which they are likely to be exposed There are two general methods of procedure in accomplishing this. Either the varieties and selections should

be grown in a plot where an artificial epidemic is produced by inoculating with all of the physiologic forms of the pathogens which are known to occur in the region for which the variety is intended, or the variety should be grown in many

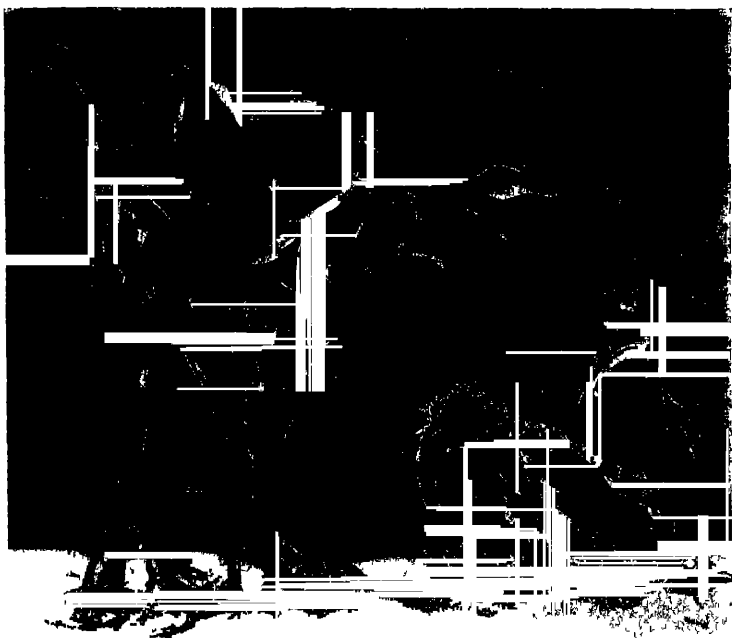


FIG. 14.—Two lines of corn inoculated with one physiologic form of *Ustilago zeæ*. Left Salmon silk (culture 1800–1925), only very small smut boils developed. Right Brown aleurone (culture 1700–1925), large smut boils and distortion of plants are shown. Of twenty-one plants inoculated in the field, twenty were killed.

different localities in the region. In the first case one brings the forms to the varieties, in the second, one hopes that he is taking the varieties to the forms. The ideal system is a combination of the two methods.

Can resistant varieties of crop plants actually be produced when there are numerous physiologic forms of many of the most destructive pathogens? It is significant that the same genetic factor may sometimes govern resistance to a number

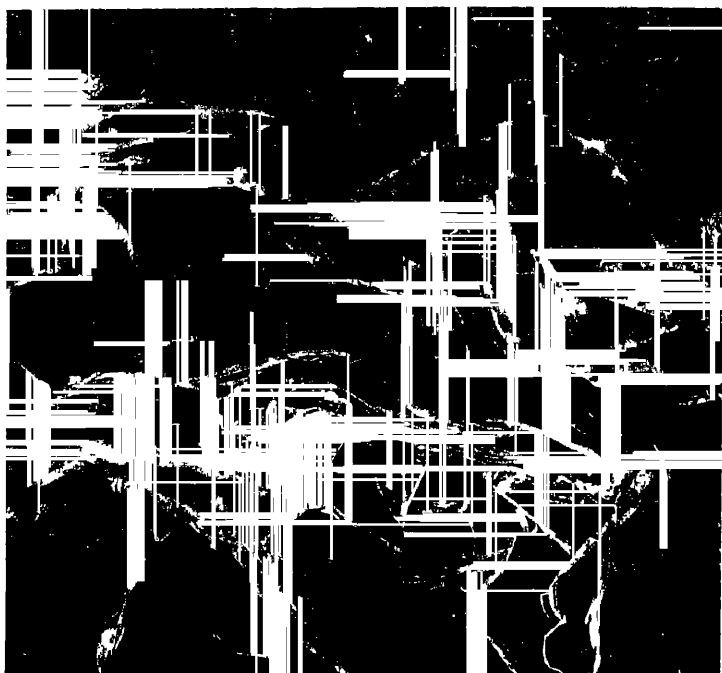


FIG 15—Two hitherto "resistant" lines of corn severely injured by a virulent form of *Ustilago zeae* from Pennsylvania. About 95 per cent of the plants were killed. Both strains had previously proved resistant to smut for several years at University Farm.

of physiologic forms. For example, Kanred wheat is immune from about twelve of the physiologic forms of *P. graminis tritici* so far described by Stakman and Levine¹²⁰ and the immunity from all of these dozen forms is governed by a single genetic

factor¹ When Kanred is crossed with susceptible varieties, immunity is dominant over susceptibility, and some of the hybrids are immune from all of the physiologic forms from which Kanred is immune By developing varieties which are resistant to a number of physiologic forms, then combining them with other varieties which are resistant to still other forms, it is possible to build up synthetically varieties which are resistant to many forms This method has been used by McRostie⁹⁰ and by Burkholder³⁰ in developing varieties of beans resistant to different physiologic forms of the bean anthracnose organism. It also is being used in breeding stem rust resistant varieties of wheat^{66*} (Fig. 16)

The following may show how the method operates It was desired to obtain a hard red spring wheat of good quality and resistant to black stem rust. Marquis, the most popular variety in the spring wheat region, is very susceptible Therefore, it was crossed with Kanred which is immune from certain physiologic forms, but which is a winter wheat, and therefore not adapted to the hard red spring region. Some of the selections from the cross had the spring wheat habit of the Marquis, and were immune from all of the rust forms from which the Kanred parent was immune But Kanred, as well as these selections from the hybrid, rusts in some years and in some regions Therefore, some of the hybrids were crossed with Marquillo which had been derived by crossing Iumillo, a resistant durum, with Marquis. The Marquillo is resistant to many physiologic forms of stem rust and has a

* For discussions of the development of varieties of wheat resistant to stem rust see Nos 1, 2, 60, 61, 62, 65, 71, and 108 in the Bibliography

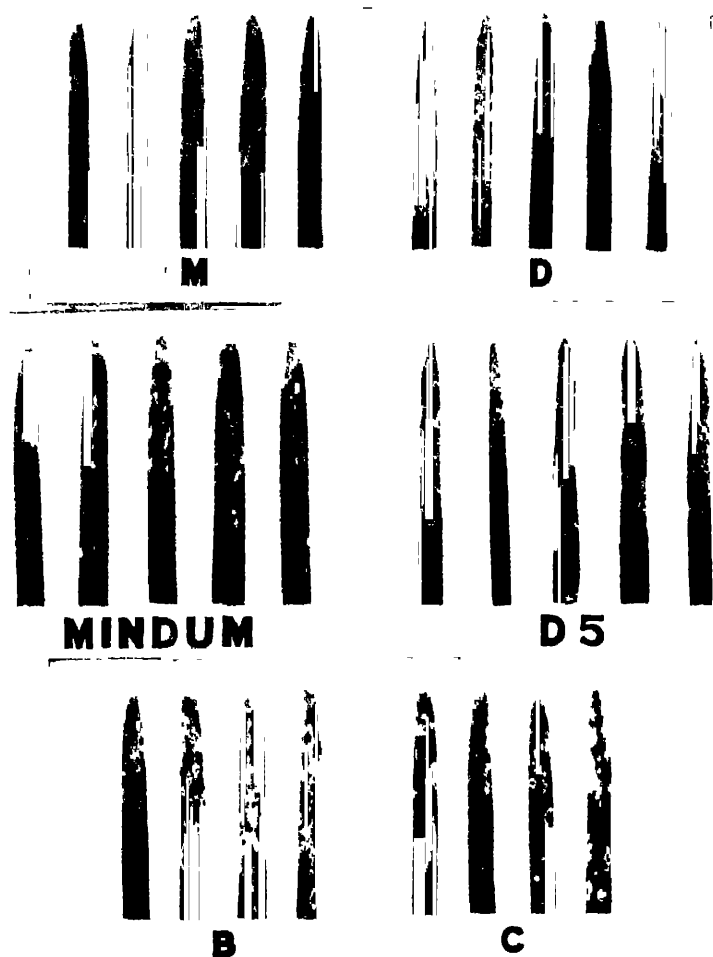


FIG. 16—The synthetic production of a desirable durum wheat resistant to physiologic forms of stem rust. Mindum, an amber-seeded durum of good quality, is susceptible to many forms of rust. Pentad, a red-seeded variety, is of poor quality but is resistant to many forms of rust. The resistance of both has been combined in a durum of good quality. Above, Mindum (M) and Pentad (D) inoculated with rust form 34. Mindum is susceptible and Pentad is resistant. Middle, both varieties inoculated with rust form 1, Mindum almost immune and Pentad susceptible. Below, two F_3 families of the Mindum \times Pentad cross inoculated with rust form 34, to which they are resistant. They are immune from rust form 1 and have amber seed.

fairly high degree of resistance in the field. The double cross is very promising. In this way, several varieties have been used as parents in the production of desirable rust-resistant varieties, and there is reason to suppose that by making the proper crosses and recrosses, varieties may be obtained which are of good commercial value, and are sufficiently resistant to stem rust to enable them to yield well in spite of epidemics.

There also is hope that varieties will be found with structural or functional characters which make it difficult for a pathogene to enter, or to develop extensively if it does enter. Such varieties would therefore be injured but little even though some of their tissues were susceptible. Certain varieties of wheat apparently have such morphologic and functional peculiarities. Hursh⁷⁰ has shown that there is so much woody tissue in the stems of some varieties that the rust cannot develop extensively even if it does get in. There seems every reason to suppose, therefore, that valuable varieties of many crop plants can be produced which are resistant to many physiologic forms.

How long will resistant varieties retain their resistance? There is nothing to indicate that a disease-resistant variety actually loses its resistance. It is true that the degree of resistance often can be influenced considerably by environmental conditions,⁷⁵ but resistance is due to genetic factors just as truly as are structural characters of plants. A fluctuation in resistance is to be expected, just as fluctuations in form are to be expected, but there is no good evidence that a variety once resistant to a certain physiologic form of a pathogene ever lost that resistance, although it most assuredly is

true that a variety resistant to one physiologic form may be quite susceptible to another form. Furthermore, it must be remembered that new physiologic forms are arising by hybridization and mutation. Therefore, it is impossible to know at any given time all of the physiologic forms to which a new variety eventually will be exposed. Some varieties have remained resistant for a long time. On the other hand, it is known that resistant varieties may remain free from attacks of disease for a number of years and then more or less suddenly become infected because of the introduction or natural development of new physiologic forms. This has been strikingly shown in the case of varieties of sorghum resistant to kernel smut ¹³⁸. For a number of years, milo, hegari, and feterita were practically immune from smut, both when inoculated artificially and when grown under natural conditions in the field. But in 1923 kernel smut was found on milo and hegari in certain localities in southwestern United States. These sorghums apparently had lost their resistance, but actually they had not.

Tisdale, Melchers, and Clemmer showed that the physiologic form on milo and hegari was different from that commonly occurring on sorghum. The original form could attack the sorghums but not milo, hegari, and feterita. The new form can attack the hitherto susceptible sorghums and, in addition, the hitherto resistant milo and hegari. And, according to Tisdale, Melchers, and Clemmer, there is some evidence that there is a third form, which can attack feterita, but not milo. The real explanation, then, of the apparent loss of resistance is that new forms of the smut fungus appeared.

Whether these new forms are undesirable immigrants from foreign countries, or whether they are natural hybrids or mutants is not known certainly. But they are here and must be reckoned with. There have been similar experiences in the past and undoubtedly will be in the future. This means that our ideas regarding resistance in crop plants probably will have to be readjusted from time to time. And it probably means also that new varieties will have to be produced from time to time in order to checkmate the pernicious activities of pestiferous pathogenes which bring up new reinforcements in the shape of new physiologic forms, to wage their incessant warfare against crop plants.

The fact of physiologic specialization need not make us too pessimistic about the possibilities of controlling diseases by the use of resistant varieties. We at least have learned something about the devious ways of many pathogenes, and can, therefore, work more effectively.

SUMMARY

I have attempted to point out, first of all, that plant diseases are tremendously important in man's struggle for existence, that a very important factor in successful agriculture is the control of these diseases, and that one of the most important factors in the control of the diseases is knowledge regarding the physiologic or parasitic specialization of plant pathogenes. It has been pointed out that physiologic forms can be recognized by their pathogenicity on certain selected plants, by appearance in artificial culture, by physicochemical reactions, and sometimes by morphology. The older idea

regarding the change of parasitic capabilities as a result of association with certain host plants has not been substantiated by more recent work. It has been shown, however, that physiologic forms probably have arisen and still are arising as a result of hybridization and mutation. The fact of physiologic specialization explains certain puzzling facts in connection with the development of plant disease epidemics, it is important in establishing quarantine regulations, in recognizing certain varieties of crop plants, and, above all, in the production of disease-resistant varieties of crop plants. The fact that there are so many physiologic forms and that new ones continually are being produced increases the difficulty of controlling plant diseases. But information regarding the essential facts in physiologic specialization is accumulating rapidly and it certainly is helpful to know what we have to contend with. Such progress as is made in many phases of plant pathology will at least rest on a firm foundation, and there is some comfort in that.

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THE RELATION OF PLANT PATHOLOGY TO HUMAN AFFAIRS*

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All species or races of wild plants are subject to diseases and pests. Evidence exhibited in the fossil remains of plants show that this was true of the flora of the earth long before man appeared in the woods. Not only the larger plants, the tree, the herbs, and the grasses are afflicted with diseases that maim and kill, but even the smallest of vegetable organisms, the bacteria, are now known to succumb by the billions to the attacks of the bacteriophage. In plants as in man and animals, certain individuals of every species, if a large enough population is considered, will be found to be immune or highly resistant to a given malady. But that any individual ever exists which is immune to all the diseases peculiar to the species is very doubtful. Disease is apparently the nemesis of living things.

Most diseases of plants are not promptly fatal, many of them are never so. Where they do not quickly kill, they may shorten the plant's natural term of life, or merely interfere in one way or another with its fullest development.

* Lecture given before the State University of Iowa, Iowa City, April 4, 1927, Academy of Medicine, Des Moines, Iowa, April 5, University of Minnesota, Minneapolis, April 6, and The Mayo Foundation, Rochester, Minnesota, April 7

Whenever man has brought a species of plant under cultivation, he has unwittingly brought along the pathogens and pests to which the species is subject in the wild state, and in most instances has by his very methods of domestication favored their perpetuation, dissemination, and distribution. As with animals, so with plants, nothing is perhaps more favorable to the epiphytotic development of diseases than crowding incident to domestication. In his migrations over the earth, man has always carried with him whenever possible his favorite plants as well as his domesticated animals, and along with them have gone their peculiar pests and pathogens. Very often in the new lands which he has occupied the pests and pathogens peculiar to the wild plants of that region have fallen on the introduced crops with destructive effect. On new species of plants which he has sent back to the homeland have often gone their natural disease producers to attack related crop plants there and work a destruction and devastation out of all proportion to that which these agents cause on native susceptible plants.

Man has always been dependent on plants for food, clothing, and in large part for shelter. Our own mythical ancestors, it will be remembered, were sheltered by the trees of a garden and fed on apples from their boughs, they covered their nakedness with the leaves of the fig. Even though primitive man ate largely flesh and clothed himself in the skins of beasts, such food and clothing were possible only because of the plant life of the earth.

Thus in the ages before man learned the art of domestication and cultivation, diseases in the wild plants on which he

subsisted must frequently have brought him inconvenience, even famine, and were doubtless at times the basic cause of his migrations, with their attendant wars and other hardships. We cannot prove this, but we may safely conclude, from events within our memory, that such must have often been the case in the dim ages of prehistoric times. We have within the last twenty years witnessed the all but complete extinction of our native wild chestnut by that oriental fungus, *Endothia parasitica*. Had this catastrophe befallen the chestnut in the days of Indian occupation of the Appalachian region, we can imagine some of the inconveniences at least which this rapid disappearance of an important source of food would have had on the Indian population.

In many of the most ancient writings of historic times are to be found references to diseases of plants and their economic effects. The Hebraic chronicles abound with records of hail, drought, mildew, rots, and insects which afflicted the crops in ancient Palestine, and tell of the hardships which these visitations of a wrathful Providence brought upon the Jewish agriculturists. In the days of Ancient Greece, diseases of crops were of such importance as to deserve the attention of the great philosopher Aristotle. His disciple, Theophrastus, the father of botany, devotes several chapters in his *Historia Plantarum* to the diseases of trees, grain, and vegetables. Rust was so destructive in the grain fields of ancient Rome that a pair of special deities, Ribigus and Rubigo, were yearly besought by ceremonies and sacrifices to avert the ravages of this disease. Pliny the second, that great Roman encyclopedist, in his *Natural History* makes

frequent mention of diseases of trees, grain, and the vine and often gives fantastic direction for their prevention or cure.

During the dark ages from the fall of the Roman Empire to the fifteenth century men seem to have been more concerned with saving their souls than in saving their crops, but in the few agricultural writings which have come down to us from this period there are items here and there which indicate that diseases continued to ravage the crops of the husbandman, and insect pests still took their toll. With a fatalism hard for us to understand our European ancestors through these centuries accepted such devastations of their fields and vineyards as the visitations of an avenging deity. They appear to have had little interest in their real nature, and as little concern for their prevention or control.

With the reawakening of western Europe in the fifteenth and sixteenth centuries came a quickening of man's interest in the phenomena of nature and a more rational attitude of mind toward their causation. The agricultural literature of the seventeenth century shows widespread and growing interest in diseases of plants in western Europe. Opinions as to their cause, it is true, were still largely dominated by the philosophies of the ancients and the inherited superstitions of the dark ages. The same was true of disease in man and animals. There was, however, a remarkable interest in, and wealth of, suggestions for their control. Most of these were, from our point of view, foolish and futile, but they indicate a generally awakened appreciation of the economic importance of these diseases to agricultural production. It is significant that, so far as we know, the first legislation aimed at the con-

trol of a plant disease was enacted during this period. The grain growers of Rouen, France, in 1660 obtained a decree requiring the grubbing out and destruction of all barberry plants as they were held to bear some mysterious relation to epiphytotics of wheat rust

It was, however, during the eighteenth century that diseases in plants first attracted the attention of scientific men as a field for research. The labors and teachings of the great Linnæus in the field of plant and animal taxonomy stimulated remarkable interest in the nature and classification of diseases in plants. The plant pathologists of this century were interested primarily in the nature of diseases as expressed through symptomatology. They laid the foundations of the terminology of modern plant pathology. Practical methods of control of diseases continued to engage the interest and attention of a growing number of educated gardeners and agriculturists. The essential principles of tree surgery, especially the treatment of wounds and cankers, were set forth by such writers as Riedel and Forsyth during the latter half of that century.

While the lay thinkers and writers on diseases of plants still continued to place responsibility for the maladies on the deity, the stars, or the weather, there was gradually formulating in the minds of the scientific workers in this field the doctrine of the autogenetic origin of disease within the living substance of the plant itself. This dogma was most fully elaborated by that botanical genius of the early half of the nineteenth century, Franz Unger, and set forth in detail in his *Exanthema der Pflanzen*, published in 1833. He held

disease to be an autogenetic disorganization of nutritional processes having their origin in the lack of certain chemical constituents of the sap. The entophytic fungi commonly found associated with the lesions on diseased plants he held to be the products of the disease, after organisms produced by a spontaneous transformation of the diseased sap and substance of the plant.

In spite of this general and growing interest in plant diseases, both among the educated growers and scientists of Europe, the early decades of the nineteenth century found the vast majority of lay folk, including land owners and peasants, largely ignorant of, or indifferent to, the tolls taken yearly from the crops of field and garden. America was still too busy clearing her forests and breaking her prairies, from which to take the bountiful harvests which a virgin soil yields, to concern herself with the paltry filchings of pest and disease. But the years were now marching rapidly toward phytopathologic events which were to affect profoundly large groups of persons in Europe and America.

The potato had been introduced into Europe from America during the sixteenth century. The opening of the nineteenth century saw its cultivation general throughout western Europe. In Germany, Holland, Belgium, Spain, Italy, France, Scandinavia, and England it had become a staple crop. In Ireland it was almost the sole source of carbohydrate food for the population. Ireland was almost one great potato patch. More than 4 per cent of all her lands, only a small part of which was under cultivation, were planted to this crop, and a majority of her 8,000,000 people were de-

pendent on the potato for their sustenance. The extent to which this plant had conquered the little green isle is reflected in the name, the Irish potato

During the decade 1830-1840, a strange malady began to make its appearance in the potato fields here and there in different countries of Europe, in northeastern United States, and the maritime provinces of Canada. The opening of the next decade found it so common and destructive as to arouse the greatest concern among farmers in many localities. In 1841 it swept the Rhine Pfalz so destructively that the Royal Scientific Academy of Munich engaged the savant von Martius to make an investigation of the disease. He published the first illustrated account of it under the name *Stockfäule und Knollenfäule*, in 1842. It was present in Belgium and Denmark as early as 1842, and by 1844 had become so prevalent that farmers' meetings were held in the latter country to discuss the calamity. Everywhere throughout western Europe potato growing was threatened by the disease. The gravity of the situation impressed itself on all the people from peasant to politician, and the scientific men of the different countries were for the first time in the history of the world called on to save the people, a strange spectacle indeed, for mankind had for centuries theretofore depended on their priesthood to ward off scourges of their gods.

In the New World the insidious malady had broken out about Boston, New York, and in Pennsylvania as early as 1842 and 1843. By 1844 it was widespread throughout northeastern United States, Nova Scotia, and New Brunswick.

The summer of 1845 came and with it the culmination of the threatened calamity. The potato blight swept the potato fields from southern Europe to the northernmost plantings of Scandinavia. In a like manner it overwhelmed the crop of northeastern United States and Canada. The devastation was complete. The vigorous green foliage basking in the late summer sunshine as it builded the starch to fill the swelling tubers in the soil below was suddenly killed and blackened as if the hot sun of late summer shining through the rain and mist had scorched and scaled it. No wonder there was widespread belief in a mysterious miasmatic influence in the atmosphere which wrought this havoc. It must be remembered that even at that late date, but a few unknown mycologists really knew of those microscopic organisms, the fungi, and fewer still believed them to have any causal relation to disease. Scarcely had the potato tops withered and died before the tubers in the earth were everywhere rotting in the soil soaked with the autumn rains. The devastation was appalling.

While the loss of the potato crop of 1845 was everywhere a calamity, the catastrophe was most complete in Ireland. The staple food supply of an entire people was destroyed by disease with a suddenness and a completeness unparalleled in the recorded history of mankind. Famine stalked in every Irish home. All day the hungry peasant dug his fields to find enough whole tubers among the soft and stinking masses to make his simple evening meal. The suffering was intense. In spite of the most generous governmental and private aid a quarter of a million Irish people died of hunger or of the fever

resulting from a lack of food. On the Continent and in north-eastern North America the loss was keenly felt even though famine was generally averted by the use of grain and other crops.

But the violence of the disease had spent itself. The years immediately succeeding that fatal 1845 saw a rapid lessening of the dangerous malady. It still appeared with destructive effect in fields and localities here and there in all potato growing countries, sometimes reaching the proportions of a general epiphytotic as in the succeeding seventies. Never, however, has it approached its catastrophic performance of 1845.

Thus for the first time in recorded history did a disease of plants engage the personal attention and concern of a large part of the earth's population. The results were numerous, far reaching, and most significant for mankind. Out of the devastation of the potato blight was born modern plant pathology with all the benefits it has already brought, and the still greater services it is destined to render mankind in his ever increasing population of the land areas of the earth.

A disaster to their chief food crop, so complete and puzzling, aroused the people of Europe to demand the discovery of its true cause and to find effective measures against its recurrence. The governments of those countries which had suffered most from its ravages appointed commissions to investigate the malady. While in some cases noted scientists were called to sit on these commissions, but little came of their deliberations, as is usual even in this day. But the stimulating call of a great problem inspired the genius of

obscure investigators grounded in the botanical sciences of that day. The Rev. M. J. Berkeley of England, who preached for a living and studied fungi for pleasure, examined the blighted leaves through his microscope and proclaimed in 1846 his confirmation of the discovery of Montagne and of Morreau, French investigators of 1845, that a fungus was at the root of the evil, a view which von Martius had suggested as early as 1842. A number of other scientists in Europe during 1845 and 1846 found the fungus constantly associated with the lesions of the disease, and gave it various names. None of these men, however, proved beyond debate its causal relation to the disease.

Their discoveries and assertions, however, gave a new impetus to researches on other well known diseases of crops, especially the rusts and smuts of grain. For more than two centuries the peasant farmers of Europe had believed in some mysterious relation between barberry bushes and the black rust of their cereals. So convincing were their observations that in Denmark war on the barberry was declared and campaigns for its eradication from field and village were vigorously prosecuted by the farmers on their own initiative. Scientists of the day began to believe and to seek a solution of the puzzle. Sir Joseph Banks espoused the theory of fungus responsibility and distributed widely about 1805 a pamphlet to farmers setting forth the evidence that rust was caused by a fungus which propagated itself by seed-like spores. One might cite the names of many others who during the early years of the nineteenth century contributed to the growing belief that rust was caused by an independent fungous parasite.

A young German student instructor² in the laboratories of the University of Tübingen published, in 1853, at the age of twenty-two a classic contribution on the rust and smut fungi. Here for the first time was set forth convincing evidence of the causal nature of the spores of these fungi in producing these diseases.

The smuts of cereals had doubtless been observed by grain farmers in ancient times. The peasants of western Europe were well acquainted with these diseases during the fifteenth and sixteenth centuries and clearly appreciated the losses which they produced. They, however, generally attributed these maladies to immature seed, weather, or some mysterious influence in the air. That the black sooty or greasy masses which took the place of the kernels were spores of a parasitic fungus was not even suspected by them. Even before the opening of the nineteenth century mycologists here and there who studied these sooty masses had discovered them to be composed of minute globose bodies, which they believed to be the spores of fungi and in some way responsible for causing the disease in the succeeding crop. Their causal relation to the disease, however, they did not prove and the beliefs of these rather obscure scientists found no general acceptance either by farmers or by the plant pathologists of the time. Even the discovery, due to the accidental sinking of a ship off the coast of England in 1660, that soaking grain in salt water would prevent the stinking smut or bunt of wheat, did not serve to clarify the scientific riddle. The practice of brining seed grain became, however, general throughout England. Thus we have here what so often has happened

since, the accidental discovery and adoption of a practical method of control long before the scientific basis for the practice is understood.

In 1861 de Bary,³ now a professor in the University of Freiburg, published his classic dissertation on potato blight, presenting conclusive evidence that it was caused by a fungus, thus establishing the belief of Berkeley and other investigators who had studied the malady during the great epiphytotic fifteen years earlier. He proved by careful observations and experiments that this fungus produced the blighting of the tops and the subsequent rotting of the tubers as well. Others quickly confirmed his observations. In 1865 he confirmed the age-old belief in a baneful relationship between barberry and wheat rust, proving it to have a sound scientific foundation in actual facts.⁴ By inoculating barberries with spores of the wheat rust he produced the well known cluster cups on barberry leaves.

Stimulated by these remarkable discoveries of de Bary on the causal relationship of fungi to disease, a host of young and enthusiastic investigators throughout Europe and America began the investigation of all sorts of diseases of plants. Robert Hartig in Germany prosecuted those studies on heart rots of trees which today constitute the basis of our forest pathology. Paul Sorauer, also a German scientist, devoted himself largely to diseases of plants in which no causal organisms appear to be responsible for the pathologic conditions, and laid the foundation of our knowledge of what is sometimes unhappily called "physiological diseases" of plants. De Bary meanwhile (1872) had gone to a professorship in the University

of Strassburg where for a decade and a half students from all over the world flocked to study under his inspiring direction

Among those who during the last half of the nineteenth century made notable contributions to the etiology of plant diseases may be mentioned the following: Woronin, a Russian, showed that club root of cabbage and turnip is caused by a slime mold, Burrill, an American, and Wakker, a Hollander, simultaneously discovered that bacteria cause diseases in plants, Rostrup, a Dane, uncovered the cause of many diseases of trees; Eriksson, a Swedish investigator, discovered biologic specialization among rust fungi; Prillieux in France and Comes in Italy laid the foundation of modern plant pathology in their respective countries Marshall Ward, an Englishman, discovered a rust fungus to be the cause of the disease which rapidly wiped out profitable coffee growing in Ceylon and the Far East. This rust gave to Brazil that supremacy in coffee production which she retains to this day

But while all these enthusiastic and exciting explorations into the causes of disease in plants were absorbing the energy and genius of the plant pathologists of the latter half of the last century, what was being done to effect their control and reduce the toll which they were taking from the farmers' crops? The answer is "little." Little in comparison with efforts being devoted to uncovering the causes of these maladies But that little was very important and of fundamental significance.

Stimulated by the discoveries of de Bary and his students, another young German, trained and experienced in the best practical agriculture of his day, turned his steps, in 1855, at

the age of thirty toward the Agricultural College of Poppelsdorf and the University at Bonn. Already familiar with the new ideas on fungi as producers of plant diseases, and inspired through study and experiments on the estates which he had managed, Julius Kuhn became imbued with the significance of those discoveries in their application to the control of diseases in crops. A year at the universities of Bonn and Leipzig sufficed to win for him his doctorate and he returned to the land to solve the practical problems which so much interested him. Two years later (1858) he published the first modern textbook of plant pathology in which, among other important contributions, is set forth the first practical method of seed disinfection by the use of chemicals, the so-called copper sulphate dip. Kuhn's method was based on experiments made by Prevost in 1807, and although more or less in use before his day Kuhn really brought it into general favor. But the great achievement of Julius Kuhn is that he was the first to apply the scientific knowledge of his day to the solution of the practical problems of plant disease control. So fundamental has been this contribution that it remains to this day the vitalizing factor in the wonderful growth and development of plant pathology. Truly this man was the father of modern plant pathology.

The downy mildew of grapes, caused by a fungus native on American wild grapes, appeared in French vineyards near Bordeaux about 1878. It spread rapidly, and its destructive effects soon threatened the wine industry of France. Alexis Millardet, a student of de Bary, had been employed by the French Government in 1876 in an effort to stay the ravages

of the *Phylloxera*, an insect pest of grapes which had earlier been introduced from America. He now turned his attention to the downy mildew problem. He soon discovered that copper in solution is very toxic to the spores of this fungus. But his efforts to find a form of this substance which was effective when applied to the vines were at first in vain. The rains washed the soluble salts from the foliage and the mildew spread unchecked.

Passing a vineyard one day near Medoc in the neighborhood of Bordeaux, Millardet observed several rows of vines next to the highway on which the leaves were still green and the grapes plump and ripening while the vines in the vineyards beyond were leafless and the fruit shrivelling from the mildew. He now noted that the healthy foliage was covered with a poisonous looking blue substance which upon inquiry proved to be a mixture of copper sulphate solution and milk of lime, sprayed by the owner on his vines to scare away thieving boys. Millardet at once saw the significance of the phenomenon and the idea of Bordeaux mixture, as we call it, was born.

Here was a colloidal copper which stuck to the foliage through the heaviest rains and still retained its effectiveness against the spores of the mildew fungus.

Millardet had already conceived the idea that a "practical treatment of mildew ought to have for its aim not the killing of the parasite in the leaves, but of preventing its development by covering the surface of the leaves with substances capable of causing the spores to lose their vitality or at least hindering their germination," and here was the substance he

sought. The following year (1882) he made his first field experiments with mixtures of copper sulphate and hydrated lime. They were most surprisingly effective and after three seasons of testing the new compound in the vineyards Millardet in 1885 gave to the French vineyardists and to the agriculturists of the world the first and most effective protectant known. Pathologists in Europe and America quickly extended the application of Bordeaux mixture to many crops, especially potatoes and fruit trees, until it came to be regarded as an almost universal cure-all for plant diseases.

Thus did these two, Kühn and Millardet, pioneers in modern plant pathology, working in the light of the illuminating discoveries of de Bary and his disciples on the causal responsibility of fungi for diseases in plants, lay the foundations of practical plant disease control.

Kühn showed the way to our present-day control of many diseases through disinfection. Disinfection of seeds, tubers, bulbs, and soil is now a practice of wide and varied application. Other disinfectants have come to largely replace the copper sulphate dip of Kühn. Geuther, a German (1895), and Bolley, an American (1897), demonstrated the value of formaldehyd as a seed disinfectant. Bolley (1891) also proved the effectiveness of mercuric chlorid as a tuber disinfectant against potato scab. Jensen, a Dane (1887), developed his well known hot water treatment for certain smuts of grain. Riehm (1913) reported the superior properties of organic mercury compounds. Darnell-Smith, an Australian, reported in 1917 his preliminary experiments on the substitution of copper carbonate dust for the copper sulphate or

formalin dips in the control of bunt of wheat, thus laying the foundation for dry or dust disinfection of seeds and tubers. This is undoubtedly the most important advance in disinfection methods made during the present century.

Following the discovery of Bordeaux mixture, a great variety of copper sprays were devised and recommended, but aside from improvements in Millardet's formula no copper protectant which can be regarded as an improvement over Bordeaux mixture has as yet been discovered. However, with all its remarkable qualities Bordeaux has its limitations as a protectant. It was soon found to be injurious to some plants, especially certain fruits like peaches and apples. Cordley, of Oregon, accidentally discovered in 1906 that lime-sulphur solution at that time used chiefly as a winter spray for San José scale could, when properly diluted, be used with safety and efficiency as a summer spray against apple scab. Wallace in New York, promptly following Cordley's lead, demonstrated its superiority over Bordeaux mixture for apple spraying. It was then rapidly substituted for Bordeaux by apple growers throughout this country and is today the standard spray for fruit.

The introduction of lime-sulphur as a summer spray marks the real beginning of activity in the commercial production and exploitation of fungicidal protectants. The salesmen and service men of fungicide-insecticide concerns have been the most important agents in bringing plant disease control measures into widespread and general use. Even the efficient state and federal extension services have not contributed more to this end. In fact, the merchandizing of fungicides

and insecticides is a necessary prerequisite for effective work by the plant pathologist and entomologist. The chief deterrent to the more extensive use of Bordeaux mixture has been the want of a commercial Bordeaux equal in efficiency to the homemade mixture. The commercial production and merchandizing of lime-sulphur concentrate were the chief factors in its rapid supplanting of Bordeaux in orchard spraying.

The extraordinary developments in plant disease control during the past two decades it to be attributed largely to two factors. First, the production and distribution of commercial protectants and disinfectants as pointed out and, second, a growing appreciation on the part of growers and the public in general of the tremendous losses incurred from insect pests and diseases in our crops.

Among the incidents which have contributed in large measure to this appreciation may be called to mind the chestnut blight which started in a nursery on Long Island about 1900, where it had been introduced from Japan, and rapidly spread westward sweeping away in a few years the fine stands of our native chestnut throughout its Appalachian range. The destruction of the chestnut on private estates about New York and other eastern cities as well as in the forests aroused not only those interested in the forest, but laymen generally throughout northeastern United States. The threat of the white pine blister rust against the white pine reforestation programs in eastern United States and against our virgin stands of five-needle pines on the Pacific slope and the costly eradication campaign against the citrus canker in the citrus

orchards of Florida during the same period have contributed greatly to the growing understanding of the power for evil which these microscopic agents of disease possess. The sudden appearance and rapid spread of the boll weevil in the cotton fields of the south, the devastation caused by the gypsy moth and brown tail in New England, the steady unchecked westward spread of the European corn borer toward the great corn growing areas of the Mississippi Valley, and the relentless annual five-mile march of the Japanese beetle since its appearance in New Jersey a few years ago have brought home to nearly every thinking person in America the appalling destruction of our food, fabric, and forest plants which insect pests are sure to effect when they are allowed to get a foothold and run amuck.

During the present century and especially in the past decade, a group of plant maladies known as virus diseases have been brought into great prominence by a host of investigators especially in this country. The first and best known of these, the tobacco mosaic, was described by Mayer, a German worker, in 1886. It was not until 1899, however, that the peculiar nature of this disease was recognized when Beijerinck showed that the causal agent, whatever it is, would pass through clay filters presumably too fine to permit the passage of any known living pathogen as, for example, bacteria or fungi.

In 1806 Judge Peters of Philadelphia described the very destructive and contagious virus disease known as peach yellows, which quickly spread north and westward to practically all the peach growing sections of Delaware, New England, New Jersey, New York, Michigan, and the peach

sections of southern Ontario in Canada. It eventually spread southwestward into Tennessee, Missouri, Arkansas, and Oklahoma. Its ravages were so serious as to arouse the greatest fear for the peach growing industry of eastern United States and rigorous measures for its eradication were embodied into law in practically all peach growing states. The losses from this disease often amounted to 50 to 95 per cent of the trees in an orchard. The first scientific investigations on the malady were made by Erwin F. Smith during the years from 1888 to 1894. Its contagious nature was clearly established, but the cause of the disease in spite of succeeding attempts to discover it remains unknown to this day. It is now generally accepted to be a virus disease.

In 1905 German pathologists first recognized and described a serious virus disease of potatoes known as leaf roll. In 1911 W. A. Orton first recognized a mosaic disease in potatoes in Germany. The following year it was found throughout the great potato producing section of Maine, 100 per cent of the plants being affected in some fields.

From this time on investigations on the virus diseases of potatoes have engaged the attention of increasing numbers of pathologists in Europe and America. The viruses of these diseases were soon discovered to be transmitted through the tubers and spread from plant to plant by sucking insects, especially aphids. That these are not recently introduced maladies of the potato is certain. Serious and widespread failures of the potato crop in Europe from 1770 to 1780 are now generally believed to have been due to these diseases. The well known running-out or degeneration of potato va-

ieties attributed by growers and agricultural investigators of a generation ago to vegetative propagation is now recognized as due entirely to these virus diseases. The loss which they cause to the world's potato crop is enormous. The reduction in yield from leaf roll alone in the average potato field probably approximates 50 per cent, while a seed stock may, under conditions favorable for the spread of the virus, become worthless in a year or two. Losses from mosaic diseases in potatoes are perhaps equally serious with those caused by leaf roll, especially in cool climates.

Virus diseases are now known in practically every cultivated crop and in many plants of the garden as well as in wild plants and weeds. Among the more serious of these may be mentioned the aster yellows recently studied by Kunkel; the curly top of sugar beets which has, during the last decade, practically driven the sugar beet industry from the Pacific coast; the sugar cane mosaic, which seriously threatens the sugar industry in Louisiana and the West Indies as well as that of the sugar producing countries of the eastern tropics; the cucumber mosaic which causes a constant and economically demoralizing shifting of the pickle producing sections of the country, the raspberry mosaic and yellows which have made berry production hazardous and unprofitable for some years, tomato mosaic and streak, which take annually heavy toll in forcing house and field. This list might be extended to include such plants as beans, lettuce, sweet peas, melons, hops, peanuts, sweet potatoes, spinach, lilies, bananas, and many others.

Investigations indicate that most if not all of the viruses of

these diseases are normally transmitted from diseased to healthy plants by insects. These diseases are doubtless of the same nature as the virus diseases of men and other animals, such as smallpox, hoof-and-mouth disease, rabies, and the like

This growing understanding and appreciation by the laymen and scientists of the threat of these diseases and pests against our material safety and prosperity has been reflected in a number of ways. Great sums of money have been annually appropriated by state and federal governments and largely wasted in futile attempts to eradicate or stay the spread of these invading pathogens and pests. As is usual in such cases, steps to combat these invaders have rarely, if ever, been undertaken until they have become thoroughly established and beyond eradication or limitation in their invasive progress. In 1912 the United States Congress enacted the Federal Quarantine Act, administered by the Secretary of Agriculture through the Federal Horticultural Board. The expressed intent of this act was to prevent by quarantine the further introduction of destructive pathogens and pests from other countries and to limit the spread of those already established within our borders. No less than sixty distinct quarantines have been promulgated to date, and great sums of money have been expended in their attempted enforcement. The net result of this program in protecting us from diseases and pests is negligible so far at least as definite evidence to the contrary is available. It has, however, acted as an all but absolute tariff wall to exclude many plants and plant products heretofore imported at prices many times less than what we must

now pay for home grown products of the same kind and often of inferior quality and more affected with diseases of various kinds. The narcissus bulb situation may be cited as a glaring example of one of the many cases in point.

However, this growing interest on the part of the public in plant protection and an increasing understanding of the problems involved has been reflected in a sane and growing development of the science of plant pathology, not only in this country but notably also in Germany, Japan, the Scandinavian countries, England, and Italy. The history of plant pathology in the United States spans less than half a century. Its rise may be roughly placed at the year 1885. It began with the establishment of the Division of Plant Physiology and Pathology in the Federal Department of Agriculture. In this division were brought together a small group of able and enthusiastic young scientists who were in the following decades to contribute greatly to the foundation of phytopathologic science in this country. Preeminent in this group was Erwin F. Smith, whose discoveries and contributions in the field of bacteriophytopathology have won for him a world wide and immortal renown in plant pathology. His work on crown gall of plants and its similarity to human cancer have directed the attention of the medical profession to the field of plant pathology as a source of assistance in solving some of the problems in human pathology.

State experiment stations and colleges of agriculture slowly at first, but with increasing frequency, took up the problems of disease in plants. Jones, in Vermont, and Stewart, in New York, demonstrated through a long series of annual experi-

mentation that Bordeaux mixture was a profitable specific for the control of potato blight. Kellerman and Swingle in Kansas, Arthur in Indiana, and Bolley in North Dakota, by brilliant series of investigations, laid the foundation for seed and tuber disinfection in this country. Orton and Webber by selection and breeding of wilt resistant cotton, melons, and cowpeas disclosed the possibilities of immunization as a principle of plant disease control. Cordley, in Oregon, and Wallace, in New York, gave us in lime-sulphur a new and powerful fungicide of wide adaptability thus initiating diversification of fungicides to meet special needs and conditions in the protection of plants against fungous pathogens. Blodgett, Reddick, and Crosby in New York, and Sanders in Nova Scotia revived and vitalized dusting as a method of applying fungicides and insecticides. Today this method bids fair to largely, if not entirely, replace spraying.

Another measure of the phenomenal expansion in the field of plant pathology is the growth of the American Phytopathological Society founded in 1909, with an initial membership of less than fifty, some of whom could be called plant pathologists only by courtesy. The society now has a membership of approximately seven hundred, practically all of whom are actively engaged in some phase of phytopathologic work.

Although plant pathologists were early employed as such in the Federal Department of Agriculture, and in a number of state experiment stations, it was not until late in 1907 that the first chair of plant pathology, that at Cornell, was established in an American university. It was antedated by but two in other countries, one in Denmark in 1904, and the

other in Japan early in 1907. Today professorships in plant pathology are to be found in nearly all our colleges of agriculture and even in universities with which no college of agriculture is connected as, for example, in the University of Chicago.

It is commonly assumed that diseases in crops are the concern of the farmer alone. It is true that diseases frequently, in fact commonly, do cause serious losses to the individual farmer. But the loss of one potato grower is usually the gain of another whose fields escape the disease, or who saves his crop by the application of some effective means of control. When potato blight, for example, sweeps one potato section and thus cuts the total crop of the country, prices of potatoes go up accordingly so that the total value of the potato crop of the land is as great or greater than what it would have been had there been no losses from the disease. It is the consumer of potatoes who pays for the toll taken by the blight, not farmers as a group. The city man, therefore, is most concerned in the devastation wrought in crops on which he depends for food, shelter, or clothing. For every two sweet potatoes which are brought him at the restaurant he pays for one he does not get. On the average one sweet potato out of three produced in the United States is lost from diseases in field or storage house. In every half-dozen cotton shirts a man buys is included the cost of a seventh one lost to diseases in the cotton fields of the South.

Estimates by the plant disease survey of the United States Department of Agriculture indicate that approximately one bean in every dozen, one apple in every seven, one peach in

every eight, one bushel of Irish potatoes in every twelve, and one bushel of wheat in every ten, are destroyed annually by diseases in these crops. That these estimates, large as they may appear at first sight, are extremely conservative and probably far below the actual losses in most cases is indicated by recent experiments and tests in the control of some of these diseases. For example. The average yield of potatoes in the state of Pennsylvania is approximately 100 bushels an acre. Yet many potato growers in the state, spraying their potatoes under the able direction of Mr. Nixon, the extension plant pathologist, grow from 400 to 600 bushels an acre annually. These remarkable yields of 300 to 500 bushels above the average of the state are attributed by Nixon entirely to spraying and the use of disease free seed. Experiments in the dusting of spring wheat for the control of rust in western Canada in 1925 gave a yield of fifty-five bushels an acre, where the average yield from undusted plots hard by was but twenty-one bushels an acre. Making allowance for the fluctuations in the severity of rust from year to year, it is safe to say that the loss from all diseases in wheat in the United States and Canada is annually little short of one bushel in every two, half the crop that each acre of wheat lands as usually cultivated and fertilized should produce. Uncontrollable weather factors such as winter killing, drought, and the like undoubtedly often reduce crops, but much of the loss commonly attributed to weather is due to diseases. Some of these losses from disease, pathologists already know how to prevent, or are well along on the way to the development of effective control measures. Many diseases, especially those

affecting roots of plants, still baffle all efforts at effective control. All must eventually yield to persistent scientific attack by men trained and devoted to their solution

The work of the plant pathologist is by no means completed when he has discovered and experimentally demonstrated an effective and profitable method for controlling a given disease of plants. He must convince the growers of the practicability and profitableness of the method he proposes and get them to put it into operation. This often requires years of effort against obstacles of a sort similar to those that delay and handicap medical men in their attempts to alleviate and banish diseases of man and animals.

Although the number of plant pathologists grows apace, their numbers are still far too few to cope with the problems pressing daily for solution. We are only entering on the era of the professional plant pathologist. As yet most plant pathologists are employees of the state or federal government. Here and there, however, one is entering into the employ of commercial concerns interested in the control of diseases of plants. Farmers' organizations, nursery growers, smelting companies, manufacturers, and purveyors of fungicides and insecticides, and the like. The day will come when the sign of the plant pathologist will stand forth in the street alongside that of the physician and surgeon. This country can and probably will profitably support eventually more plant pathologists than physicians of medicine and surgery. For what will it profit us if all the ills and diseases of the human race be banished and we then face starvation because of diseases and pests in our food plants?

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SOME ASPECTS OF CELLULAR PHYSIOLOGY*

WINTHROP J V OSTERHOUT

I may take it for granted that in this great institution, so active in research, everyone understands that advance in the art of healing eventually depends on our knowledge of the properties of protoplasm. It therefore seems appropriate to consider today one of the most important of these properties, selective permeability.

Living protoplasm synthesizes certain substances which are necessary for its existence and which are prevented from diffusing out of the cell because its surface is sufficiently impermeable to prevent serious loss. But at the same time it admits the raw materials out of which these useful products are manufactured and it also permits the escape of certain excretions. The surface of the protoplasm is therefore said to have selective permeability and it is easy to see how this may control all the activities of the cell. Its study is of great importance as it is one of the best ways of studying the nature and behavior of protoplasm. It also has a direct bearing on such practical matters as the absorption of food and drugs, the physiology of excretion and secretion, and on certain pathologic conditions, for there is evidence to indicate that in many cases abnormally great permeability is responsible.

* Lecture given before The Mayo Foundation, Rochester, Minnesota, May 3, 1927, and University of Minnesota, Minneapolis, May 4

for the penetration of bacteria into tissues and the entrance of the proteins which induce anaphylaxis and hay fever

Plant cells offer especial advantages for the study of this subject and we are indebted to the botanists for recognition of the fact that the living cell acts as an osmometer whose behavior can be imitated by covering the end of a glass tube with bladder tissue and placing a solution of sugar inside, this absorbs water in much the same way as a living cell. For further progress quantitative experiments were needed and these began with the plant physiologist, Pfeffer, who found that a suitable osmometer gave pressures greater than those of the living cells he was studying. He observed that the pressure increased by about $\frac{1}{175}$ on raising the temperature one degree centigrade. Another botanist, de Vries, mentioned this to van't Hoff to whom it gave the suggestion that substances in solution behave like gases, for the latter show exactly the same increase of pressure with rise of temperature. This idea was developed by van't Hoff in a manner which proved to be of great importance for physical chemistry and for biology.

De Vries found a simple method of measuring the osmotic pressure of living cells. If we employ a solution whose osmotic pressure is slightly greater than that of the cell, the latter loses water (this is known as plasmolysis), and by observing in what solutions this occurs, the osmotic pressure of the cell can be estimated from that of the applied solution. If the substance contained in the applied solution penetrates the cell it will raise its osmotic pressure and, in consequence, the cell will reabsorb some of the water it has lost. It was thought

that in this way one might ascertain what substances penetrate. Unfortunately, the cell is always more or less injured by plasmolysis, and such cells can no longer be considered as normal: This fact has been largely overlooked (partly because the injury is often only temporary) and much confusion has arisen in consequence.

Overton employed this method in an extensive series of researches and endeavored to clarify the situation by applying the principles of physical chemistry. He attacked a problem of great interest, the nature of the semipermeable surface of the cell. It is evident that this determines what substances can enter the cell, and that on its composition the whole metabolism of the cell may depend. Pfeffer and others had suggested that it consists of protein, but the first systematic investigation was made by Overton. It had been suggested by Quincke that the outer layer of the cell consists of a film of oil. Overton came to a similar conclusion, substituting for "oil" the term "lipoid," which includes such substances as lecithin and cholesterol.

Overton concluded that no substance can enter the cell unless it is soluble in lipoid. The most important evidence which he brought forward may be summarized as follows:

1. Salts (which are, as a rule, insoluble in lipoid) are unable to enter the cell.
2. Dyes insoluble in lipoid do not enter the cell, but lipoid-soluble dyes penetrate readily.
3. Organic substances (including such anesthetics as ether and chloroform) penetrate and affect the cell the more readily, the more soluble they are in lipoid.

Meyer and Overton sought to formulate a theory of anesthesia on this basis, but it must be remembered that lipoids inside the cell may play an important part (this they left out of consideration)

Overton's hypothesis has been a valuable stimulus to investigation, but it has become increasingly evident that for real progress we must have accurate measurements of the amount of penetration under normal conditions, and many attempts have been made to supply this need. The penetration of dyes would seem to offer a promising method, but it is beset with many difficulties, some of which will be discussed. The penetration of acids and alkalies may be observed in cells containing indicators, but the applied solution is very likely to injure the cells. Effects on metabolism have been studied, but it is quite possible that they may be produced without actual penetration. In some cases we may observe with the microscope changes within the cell produced by penetrating substances, but this has not as yet led to quantitative results.

A method having many possibilities is that of measuring the electrical resistance of the protoplasm. This is low when ions penetrate freely and correspondingly high when they enter with difficulty. Under normal conditions the permeability to ions is too small to be measured in this way, but this method is very valuable in detecting changes due to abnormal conditions. We find, for example, that when the cell is injured its permeability increases and its electrical resistance diminishes until the death point is reached, after which there is no further change. This process can be followed from

moment to moment so that we can tell when it is half dead, and so on, and this enables us to subject it to mathematical analysis, and to study the dynamics of death

The fall of resistance enables us to measure the amount of injury. We find, for example, that in some cases in which the injury is not more than 5 per cent, complete recovery is possible when the injurious agent is withdrawn, but when the injury is greater, recovery is incomplete. This is of especial interest, since in physiologic literature it seems to be generally assumed that when recovery occurs it is always complete, or practically so, as if it obeyed an "all or none" law. But it is evident that partial recovery may be easily overlooked unless accurate measurements can be made. This fact may serve to illustrate the importance of quantitative methods in the study of these fundamental problems.

When plant material comes into the laboratory its resistance can be measured and we can tell whether or not it is in normal condition, and the figures we obtain enable us to predict the approximate length of life of each lot of material under laboratory conditions. We thus arrive at what may be called a measure of vitality or normal condition. It is evident that these measurements may enable us to place on a quantitative basis such conceptions as vitality, injury, recovery, and death.

The measurements also throw some light on the question of antagonistic salt action. As is well known, blood and sea water are balanced solutions in the sense of Loeb, since the toxic effect of each salt is antagonized by other salts (which may themselves be toxic when acting alone). In the case of the marine plant *Laminaria* we find that pure sodium chloride

at the concentration found in sea water is very toxic and causes a rapid increase in permeability with a corresponding decrease in electrical resistance. On adding a little calcium this is largely checked for the calcium has an antagonistic action. If the plant is exposed to a solution of pure calcium chloride we find that the resistance does not fall, but, on the contrary, it rises rapidly until a maximum is reached, after which it begins to decrease. In this case it is evident that the antagonistic salts have at the start opposite effects on the electrical resistance, and when they are combined in the proper proportions, these effects mutually counteract each other.

All of these facts may be brought under a single point of view by means of a theory which enables us to predict the results quantitatively. This theory assumes that the electrical resistance of the protoplasm depends on the quantity of a substance present in the surface, and that there is a series of reactions which result in the formation and decomposition of this substance. Under normal circumstances it is formed as fast as it is decomposed, so that it remains constant in amount, but when pure sodium chloride is applied its decomposition is catalyzed more than the formation, so that it decreases (the application of calcium has the opposite effect). When the toxic agent is withdrawn the normal course of events will be resumed and recovery will be complete unless the injury has gone so far that the parent substance (from which the various reactions start) has been diminished, in which case recovery will be partial.

The theory enables us to predict quantitatively what will happen, for example, when material is transferred from sea

water to a solution of sodium chloride, then to one of calcium chloride, then to a mixture of the two, and then back to sea water. It, therefore, serves a very useful purpose and will retain its value even if the assumptions on which it is based should be given up.

It is of interest that this theory regards the death process as one which is always going on even during the active growth of the cell; the only effect of the toxic agent is to catalyze some of the reactions more than others, without necessarily introducing any new reaction. In the same way such diverse phenomena as stimulation, differentiation, and development might be accounted for. The theory, therefore, provides a scheme which might include a great variety of normal life phenomena; and disease might be conceived of as producing its effects without introducing any new reaction, but merely by accelerating or inhibiting certain reactions.

Let us now return to the question of determining what penetrates under normal conditions. The obvious way to proceed is to place the cells in a given solution and, afterward, to analyze the cell contents to see what has penetrated. But with ordinary tissues this is not satisfactory, because if we crush the tissue to extract the cell contents, reactions are set up which alter the cell sap, and substances are also included that are outside the protoplasm or adhere to its surface so that the sap obtained is by no means in a normal state. These difficulties may be obviated by using very large cells from which sap may be obtained unchanged by piercing the cell with a capillary tube, or by cutting the cell open and expressing the sap by gentle pressure. I have found multinucleate cells suitable for

this purpose in the marine alga *Valonia* (5 cm or more in length) and in the fresh water plant *Nitella* (10 cm or more in length)

The first thing which strikes us in *Valonia* is the astonishing difference between the content of the cell and the surrounding sea water, as shown in Table 1. The cell consists of a delicate film of protoplasm (only a few microns in thickness), outside this is the cell wall imbibed with sea water and inside of it is the cell sap. In the sea water sodium predominates, in the sap it is largely replaced by potassium which has a concentration about forty times as great as in the sea water outside. It is evident that there is a trapping mechanism which causes the accumulation of potassium, and at the same time there must be an excluding mechanism for keeping out magnesium and sulphate, neither of which occurs inside the cell (except possibly in traces), although they are present in the sea water. This illustrates in a striking way the power of selective permeability to control the composition of the interior of the cell.

TABLE 1
MOLECULAR COMPOSITION EXPRESSED AS PER CENT OF HALIDE
(Cl + Br)

	Bermuda sea water	Sap of <i>Valonia</i> <i>macrophysa</i>	Sap of <i>Halicystis</i>
Cl + Br	100 00	100 00	100 00
Na	85 87	15 08	92 80
K	2 15	86 24	2 58
Ca	2 05	0 288	1 36
Mg	9 74	Trace?	2 49
SO ₄	6 26	Trace?	Trace?
Organic matter parts per thousand		1 433	2 09

In order to find out if this extraordinary difference is necessary to the life of the cell the experiment was made of extracting sap from a number of cells and then placing living cells in it so that the protoplasm was in contact with the same solution on both sides. Such cells promptly died. This phenomenon deserves further study. It seems possible that if the cell sap is toxic when applied to the outside of the cell this may help to explain certain effects of shock in animals following injury to tissues which sets free cell sap (in a more or less altered condition) so that it comes in contact with normal cells in other parts of the body.

Since the cell sap consists almost entirely of chlorides of potassium and sodium with little or no calcium, it is not a balanced solution in the ordinary sense of the word. This raises an interesting question for investigation.

The discrepancy between the composition of the sap and the sea water recalls the case of human erythrocytes inside of which potassium predominates although sodium predominates in the blood which surrounds them. On the other hand, the erythrocytes of certain animals contain more sodium than potassium and this is paralleled by another marine organism, *Halicystis* (Table 1), which resembles *Valonia* in appearance and which has more sodium than potassium in its sap.

Let us now return to the question of permeability to ions. The experiments indicate that the penetration of ions is exceedingly slow, as is illustrated by an experiment in which was added to nine parts of sea water one part of cæsium chloride (of the same halide concentration as the sea water). This increased the specific gravity so that the cells which normally

rest on the bottom rose to the top and continued to float for more than a year. If during this time they had absorbed any considerable amount of cæsium they would have sunk, but the only ones to do so were those that were obviously injured.

If, on the other hand, a little ammonium chloride is added to the sea water, the cells gradually rise to the surface where they float, retaining their normal appearance. Analysis shows that the sap contains ammonium chloride and that its specific gravity is lessened.

It seems to be largely ammonia or ammonium hydrate that enters, and this brings up the question of the behavior of weak bases and acids. With weak electrolytes we expect that at equilibrium the concentration of undissociated molecules will be the same inside as outside (providing the activity coefficients are equal) and that the ionic activity product (or chemical potential) inside will equal that outside. If molecules alone are able to enter we shall expect by increasing the external concentration of molecules to increase to the same degree both the final concentration inside and the amount entering in a given time so that the velocity constant of the time curve will not be changed. If there is any penetration of ions (or rather of ion groups since ions of one sign must be accompanied by those of the opposite sign except when there is an exchange of ions going in opposite directions) the concentration at equilibrium in the vacuole will be the same as if no ions entered but the velocity constant will increase and this will afford a measure of the penetration of ion groups. In other words, the distribution at equilibrium of weak electrolytes between the vacuole and the external

solution will not tell us whether or not ions penetrate, but the velocity constants may do so.

That ions penetrate very slowly or not at all is indicated by the experiments of Dr Blinks on *Valonia*, which show that the electrical resistance of the living protoplasm to direct current is extremely high. Whether this implies impermeability to both positive and negative ions or only to those of one sign is not yet clear, but in any case it means that there can be very little actual penetration of ions, for ions of one sign cannot penetrate alone (except when ions exchange going in opposite directions through the surface).

But if ions are unable to penetrate freely how can the sap contain so much salt? It seems possible that ions may unite at the surface of the protoplasm and pass through as undissociated molecules which again dissociate on the other side.

If we assumed that potassium hydrate is able to penetrate in this way (or in dissociated form) it is evident that it must continue to do so as long as the inside of the cell is more acid than the outside (the hydrogen-ion concentration value of the sap is about 5.6 and that of the sea water about 8.1). Presumably the chief acid inside is carbonic acid, and so we should expect an accumulation of potassium carbonate and bicarbonate. This would also be true of sodium hydrate, but we must assume that it penetrates less rapidly so that potassium predominates inside the cell.

It is evident that if some ions enter more rapidly than others (no matter whether they penetrate as ions or they combine at the surface to form molecules and so pass through and

dissociate on the other side), certain constraints are present which are characteristic of the Donnan principle and they must operate to force carbonate and bicarbonate ions out and chloride ions in until the ratio of carbonate to chloride and bicarbonate to chloride becomes the same inside as outside. Potassium must, therefore, tend to accumulate in the form of potassium chloride, as is actually the case. Hence as the cell produces carbonic acid, potassium hydrate enters and is transformed to potassium chloride. This would also explain the well known connection between the rapidity of growth and rapidity of production of carbon dioxide, as well as the fact that the rate of absorption of salts increases with the rate of growth.

The question arises whether the accumulation of potassium can be accounted for in some other way. The Donnan principle states that ions which do not pass through a membrane affect the behavior of those that do, so that if there are such ions on one side of a membrane they attract those of opposite sign (so that the concentration of the latter tends to become greater than on the opposite side of the membrane), and repel those of the same sign (so that their concentration tends to become less than on the opposite side of the membrane). This may be expressed by the equation $C_i A_i = C_o A_o$ in which C_i and A_i represent the concentrations inside the membrane of the cation and anion, respectively, of a pair of diffusible ions, C_o and A_o are the corresponding concentrations outside. In order to explain the accumulation of potassium on this basis alone we should have to assume a great excess of indiffusible anions inside over those outside and there is no evidence that

this is the case (especially since they would have to be organic and the amount of organic matter in the sap is very small). Moreover, the Donnan principle would require that at equilibrium all penetrating cations should have the same ratio to each other inside as they have outside, which is certainly not the case. Moreover, if potassium is forty times as concentrated inside as outside we should expect chloride to be forty times as concentrated outside as inside.

These difficulties do not exist in applying the Donnan principle combined with the assumption of unequal permeability. If we suppose that sodium and potassium ions penetrate very slowly as compared with chloride, carbonate, and bicarbonate ions, we postulate much the same sort of constraint as if sodium and potassium ions did not penetrate at all, and we may assume that the diffusible ions of hydrogen, chlorine, bicarbonate, and carbonate, enter freely. They could pass through the surface as ions or as undissociated molecules (of hydrochloric acid, carbon dioxide, or carbonic acid) which dissociate on the other side, the result being precisely the same as far as the Donnan principle is concerned. The tendency would then be for bicarbonate, carbonate, and chloride ions to have the same ratio inside as outside, which is all we need to account for the facts (equilibrium is not reached since the cell is continually producing hydrogen, carbonate, and bicarbonate ions).

If we come to the conclusion that it is mostly undissociated molecules which penetrate it does not follow that all undissociated molecules enter with equal facility. As a matter of fact we find that alcohol penetrates very rapidly while sugar

enters with extreme slowness. Such differences suggest that the penetrating substance must combine chemically with some constituent of the protoplasm or be soluble in it in order to enter. Even if such a constituent were present in the outer layer but lacking in one of the deeper layers, the substance would be unable to enter freely with the vacuole of the cell.

In order to study penetration quantitatively, it is desirable to employ a substance which enters quickly, is not toxic, and which can be measured rapidly and accurately. Such a substance is found in the basic dye brilliant cresyl blue, the penetration of which has been studied with great care by Miss Irwin. The time curve of penetration follows the course of a monomolecular reaction with a rather high temperature coefficient. It is found that the more alkaline the solution, the greater is the penetration, which suggests that the amount of free base inside tends to equal that outside. But on this basis we should not expect much penetration in the case of methylene blue. Nevertheless, methylene blue is used as a vital stain. A spectrophotometric examination of cell sap free from contamination explains the discrepancy by showing that it is principally azure B (a homologue of methylene blue) which is found inside and this contains relatively so much more free base than methylene blue that we might expect it to penetrate more from alkaline solutions.

Such a discovery should put us on our guard against drawing conclusions regarding penetration, hydrogen-ion concentration value or the oxidation reduction potential as inferred from the behavior of dyes unless it is possible to ob-

tain sufficient cell sap (free from contamination) for proper spectrophotometric analysis

It may be added that in many cases the cell appears deeply stained when viewed from the outside, but examination shows that there is no dye in the sap, the stain being confined to the cell wall or the outer surface of the protoplasm; in the case of smaller cells this might easily lead to misconceptions. Another source of error is change in the dye after entrance, which cannot easily be detected in the case of cells of ordinary size

When a cell has been stained with brilliant cresyl blue the dye comes out much more rapidly in an acid than in an alkaline solution. This is to be expected since in the former case, dye molecules reaching the outer surface are at once transformed into the dissociated form which increases the diffusion gradient and hastens the process. The mathematical theory for this has been satisfactorily worked out.

Further studies have shown that the rate of penetration depends on the condition of the vacuole and of the protoplasm as well as on that of the external solution.

A discovery of very great interest is the observation that treatment of the cell with salts previous to placing it in the dye may completely change its behavior without seeming to injure it in any way. Thus in the case of *Nitella* treatment for a few minutes with 0.001 M sodium chloride (a concentration very much less than that found in the cell sap) changes the cell so that when subsequently placed in dye (even after washing thoroughly in distilled water) the rate of penetration is reduced to about half. This effect seems to be produced by all

salts with monovalent cations and to be antagonized by those with divalent or trivalent cations (which by themselves seem to have no effect). This gives us for the first time a direct and accurate method of studying reversible changes of permeability caused by salts without any apparent injury. This subject merits careful and intensive study.

Let us now return to a consideration of the structure of the protoplasm. There are two protoplasmic surfaces, the outer in contact with the sea water, and the inner in contact with sap. The question arises whether these are identical in properties. It would seem not, for we must suppose that magnesium must penetrate the outer surface in order to form the chlorophyll found in the protoplasm and it would also seem probable that sulphate must enter since sulphur is a general constituent of protoplasm. But since neither of these substances is found in the sap, it would look as though the inner surface were impermeable to them.

Striking evidence for the correctness of this view is obtained by measuring potential differences. This is done by inserting a capillary filled with sap into the cell which continues to live impaled upon the capillary.

We can then lead off from the inside to the outside of the protoplasm. If sap is placed in contact with the outside we have the chain



Since this is symmetrical we should not expect any electromotive force, but a considerable amount is actually found. Hence it may be assumed that the chain is

sap		X		W		Y		sap
(exterior)								(interior)

in which X represents the outer surface, Y the inner surface, and W the remainder of the protoplasmic layer. If X and Y differ in composition, we can account for the potential differences observed in these experiments.

If it is possible that a film of protoplasm only a few microns thick contains layers of such different properties as appears to be the case with X and Y it is evident that we must revise our ideas regarding the forces which determine the structure of protoplasm. The idea that the surface layers of protoplasm are formed by the migration into the surface of substances which reduce surface tension would lead us to expect that both surfaces would be alike and this should also be true of any other surfaces, and we must bear in mind that other surfaces exist, for in both animals and plants there are cases in which different kinds of vacuoles exist in the same cell and it is possible that in ordinary cells each of the different inclusions in the cytoplasm is in contact with a different kind of surface, and there are also the surfaces of nuclei and of plastids, as well as of mitochondria.

The evidence therefore indicates that the outer surface X is more permeable to certain substances than the inner layer Y. It would seem from certain experiments, especially those carried out by Dr. Blinks, that at least one of the layers in *Valonia* (probably Y) is impermeable to ions since the electrical resistance of the protoplasm to a direct current is exceedingly high.

These studies lead me to look on the semipermeable membranes of the cell as even more important than I supposed. In certain cases it seems quite possible that in a cell immediately after death the same substances may be present and the same reactions go on as just before it was killed. But the difference between the living and the dead state is very marked in respect to the semipermeable surfaces; after death, they lose their selective power, and the internal and external solutions begin to mingle. When the semipermeable surfaces no longer safeguard the privacy of the chemical processes of the cell, the vital activities are unable to continue in normal fashion. The difference between a cell immediately before and just after death might, therefore, be essentially a difference in the structure and chemical composition of the semipermeable surface.

Since such semipermeable surfaces are not confined to the exterior of the cell, but exist also at the boundaries of nuclei, plastids, microsomes, and other structures in the cell, it may be that the chief advantages of cell division (as well as of the differentiation of cell organs) is to provide such surfaces and thereby to segregate various vital activities.

In this connection we may recall Loeb's declaration that the colloidal properties of matter can be manifested only where semipermeable surfaces exist. A study of these surfaces is important for chemistry, as well as for biology and especially for medicine, since by altering the conditions of such surfaces we may control the activities of the organism.

These and other studies now in progress have changed our

point of view, and are bringing a wealth of fresh problems. Although some of these may be insoluble in the present state of science, we ought at least to see how far we can go. The whole subject is so important that we cannot afford to neglect anything which may help us to understand the mechanism of these fundamental activities of the cell.



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